STUDIES OF BEHAVIORAL AND PHYSIOLOGICAL BASES OF GENETICALLY CONTROLLED EPILEPTIFORM SEIZURES IN DOMESTIC FOWL

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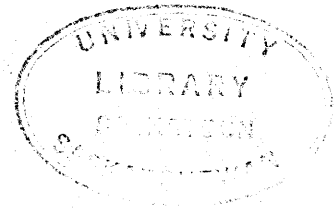

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ABSTRACT

The present studies are an attempt to obtain further information on the behavioral and physiological bases of epileptiform seizures in chickens, caused by the autosomal recessive gene epi.

The stock used in these studies was a mongrel population obtained from crosses of Fayoumi, in which the gene was first discovered, with several other breeds. Birds used in these studies were obtained from three successive generations produced from this mongrel population. A total of 2025 birds which consisted of 1603 epileptic, 120 carrier, 105 normal, and 197 non-epileptic birds (Epi-) were used. They were obtained from 48 hatches over a 2½-year period, and were studied between one day and 104 weeks of age. Some individuals were used in more than one experiment.

Seizures could be induced from epileptic chickens by heat, complex sound and photic stimulation. Intermittent light stimulation (ILS) was the most satisfactory seizure-inducing stimulus.

The response of epileptic chickens was found to be affected by age and by ILS frequency. The seizure susceptibility and incidence of complete seizures were relatively high at one day, decreased sharply during three to seven days, and increased rapidly again after seven days

of age. Incomplete seizures were not common and were found most often in birds tested between three days and 26 weeks of age. Seizures were mostly induced by less than 60 seconds of ILS and usually lasted less than 60 seconds, except in day-old chicks which tended to have prolonged seizures. Seizure latency decreased and the frequency of seizures of short duration increased gradually with age.

The most effective ILS frequency for inducing seizures in epileptic chickens ranged from 10 to 20 flashes per second (fps). Birds tested with low ILS frequencies tended to have longer seizure latency than birds tested with high ILS frequencies. Seizure duration did not seem to be affected by ILS frequency.

No differences were found between male and female chickens in seizure susceptibility, incidence of complete and incomplete seizures and seizure latency, but males tended to have longer seizures than females.

No prominent effect of parental genotype was found on the response of epileptic chickens to ILS.

Prolonged ILS during a seizure had no effect on seizure duration or on the duration of post-seizure depression which usually lasted less than ten minutes, but it significantly increased the incidence of post-seizure depression.

Prior exposure of epileptic chickens to ineffective

ILS and to heat stress did not affect susceptibility of birds immediately exposed to effective ILS. Seizure susceptibility diminished significantly in day-old chicks which had been previously subjected to cold stress, and in birds which had been previously subjected to emotional disturbances.

The resting period needed for day-old chicks to produce a second seizure was much shorter than in birds at older ages. As many as eight successive seizures could be induced from birds if effective ILS frequencies were used and the resting period after a seizure was sufficient.

Expressivity of the epi gene was complete when ILS was used for seizure induction. Sex distribution in epileptic and carrier chickens was normal. Presence of the epi gene had no effect on fertility, embryonic mortality and hatchability.

The resting EEG of epileptic chickens was characterized by relatively slow waves with high amplitude as compared to the EEG of carriers and normals. Abnormal spiking waves with frequency identical to the stimulus frequency were obtained from epileptic chickens during ILS prior to the onset of severe clonic convulsions, but were not found in the EEG of carrier and normal chickens. Carrier and normal chickens did not differ much in wave frequency and amplitude in resting EEG and in EEG during ILS.

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1. INTRODUCTION

Epilepsy is a symptom of an underlying brain disorder. It is relatively common in humans. Approximately 15 million people, or 0.5 per cent of the world population suffer from epilepsy (Jasper et al., 1969). The affected person was believed by ancient people to be visited with an evil spirit or a divinity during a seizure. This superstition remained for centuries, until about 400 B.C. when Hippocrates made known his belief that the site of origin of epileptic seizures was in the central nervous system. The development of the electroencephalographic technique (EEG) by Berger in 1929 (Lennox, 1946) for the study of brain waves was a major advance. Evidence obtained from brain wave studies has led to the recognition of the fact that epileptic seizures are due to uncontrollable excessive discharges of neurons in the central nervous system which are accompanied by sudden disturbances of function of the body or mind, such as loss of consciousness, uncontrollable tonic (a state of continuous involuntary muscular contraction) or clonic (a state of involuntary contraction and relaxation of a muscle occurring in rapid succession) muscular contractions, disorders of sensation, emotion and visceral function, and many other symptoms (Hoch and Knight, 1965; Jasper et al., 1969; Lennox, 1960; and others).

Although from the time of Hippocrates, interpretations of epilepsy based on superstition were consistently opposed, the treatment of epilepsy suggested by many physicians was still infused with superstition. No effective method or drug was found to control or cure epilepsy until 1857, when Sir Charles Locock (Robb, 1965) reported the first highly successful use of bromides, which were found to have great value in reducing the frequency of seizures. Bromides were used in the treatment of epilepsy for many years, but were found to produce side effects such as drowsiness and disfiguring eruptions of the skin if large doses were used on the treated patients. The introduction of phenobarbital in 1912 by Hauptmann (Kallmann and Sander, 1965; Robb, 1965) provided a substitute for bromides. Phenobarbital is more effective in controlling convulsions (grand mal) and produces less serious side effects (Lennox, 1946; Robb, 1965). In the last four decades, many effective drugs have been developed by workers in this field for controlling various types of human epilepsy. In recent years, the most commonly used anticonvulsants for the treatment of human epilepsy have been barbiturates (e.g. luminal, mysoline), hydantoinates (e.g. dilantin, mesantoin), oxazolidiones (e.g. tridione), succinimides (e.g. milontin, zarontin), and others such as acetezoleamides, and acetyureas (Robb, 1965; Schmidt and Wilder, 1968; Sutherland and Tait, 1969).

Many problems still exist in the treatment and in the understanding of epilepsy and there is need for further research. There are many reasons why the use of human epileptic patients for extensive studies of human epilepsy is not feasible. Animal models are more suitable and have some advantages over human subjects for basic studies. Uniformity of animals and numbers of animals are easier to provide for experimentation than are human subjects. It is too risky to use human beings for testing newly invented drugs, new treatments or proving new hypotheses about epilepsy. By using animal models, many types of experiments can be carried out with fewer restrictions and limitations.

Two kinds of animal models have been used for studies of human epilepsy. One of these consists of normal animals with experimentally induced epilepsy and the other consists of animals with genetically controlled epilepsy.

Epilepsy or conditions similar to epilepsy have been successfully induced in normal animals (e.g. primates, dogs, cats, rabbits, rats and mice) by a variety of methods such as by electric shock, nutritional deficiency, injection of various drugs or other substances, application of chemicals to the cerebral cortex, and freezing of the cerebral cortex (Blum and Liban, 1960; Blum et al., 1961; Carrea and Lanari, 1962; Dow et al., 1962; Hoch and Knight, 1965; Kopeloff, 1960; Kopeloff et al., 1942, 1965; Lockard and Barensten, 1967; Robb, 1965; Schmidt et al., 1959;

Ward et al., 1969; Wilder, 1968). Unfortunately, epileptic seizures produced by the use of these methods are often limited in time or terminate with death of the animals, as compared to human epilepsy which is characterized by recurrent attacks for an extended period of time (Kopeloff et al., 1965).

Genetically controlled epilepsy has been found in baboons (Killam et al., 1966), in cattle (Atkeson et al., 1944), in goats (Hooper, 1916; Lush, 1930), in dogs (Croft and Stockman, 1964; Ebertart, 1959), in rabbits (Nachtsheim, 1939, 1940, 1941, as quoted by Gruneberg, 1947), in rats (Finger, 1943; Griffiths, 1942; Maier, 1943; Maier and Glaser, 1940), and in mice (Collins and Fuller, 1968; Dice, 1935; Frings and Frings, 1953; Fuller et al., 1950; Fuller and Sjursen, 1967; Ginsburg and Starbuck-Miller, 1963, as quoted by Ginsburg and Sjursen, 1967; Watson, 1939, as quoted by Gruneberg, 1947; Witt and Hall, 1949). Some of these, for instance baboons, rabbits, rats, and mice, have been used extensively in studies of epilepsy. Epileptiform seizures in these animals are hereditary and are believed to have polygenic inheritance. The patterns of seizures are in many aspects similar to those found in humans.

Recently, a new mutant causing epileptiform seizures in domestic fowl has been reported by Crawford (1969, 1970). The seizures in these chickens occur throughout life and are in some ways similar to the grand mal

seizures in human epilepsy. The author described the seizures as follows: "The seizures vary in intensity and duration within individual birds..... The first sign is extreme alertness, the bird standing motionless with feathers of the head region flattened to the body. It begins to make violent pecking motions with the beak and to vocalize loudly. The bird suddenly runs, sometimes in circles, sometimes in a straight line, oblivious of objects in its way. It staggers, loses coordination of legs and wings and falls to the floor. Legs and wings thrash violently, and usually the head is thrown back. During this stage, mortality can occur from choking on debris. A coma of variable duration follows, usually with muscles relaxed. The bird regains its feet and remains motionless for several minutes during which time it appears to be totally blind or refractory to visual stimuli. Complete recovery follows."

The epileptiform seizures in chickens are controlled by a single autosomal recessive gene (Crawford, 1969, 1970). There are great advantages in using these birds for laboratory studies of epilepsy. A large number of positively affected chicks can be provided at any time of the year by simply mating affected individuals. For ontogenic studies, affected embryos are readily available. Because only one gene is involved, the affected birds have no genetic variation among themselves for this particular

trait. It may be very useful to use these birds for pleiotropic, linkage or enzyme studies. Other advantages of using chickens for experimental study are that they are easy to handle in the laboratory and are economical to maintain.

The purpose of the present study is to extend knowledge of the behavioral and physiological bases of epileptiform seizures in the mutant chickens described by Crawford (1969, 1970) in the hope that these chickens may become a valuable animal genetic model for further study of epilepsy in humans.

The study consisted of four parts.

Part I consisted of four experiments which were conducted in an attempt to obtain a satisfactory method for inducing seizures in genetically susceptible chicks. These consisted of (1) the response of epileptic chickens to heat and cold stimulation; (2) the effects of two different types of olfactory stimulation; (3) the response of epileptic chickens to two different types of auditory stimulation; and (4) the effects of various environmental light conditions including intermittent light stimulation.

Part II consisted of six experiments designed to study epileptiform seizures induced by intermittent light stimulation. The six experiments concerned (1) the effects of age and flash frequency of intermittent light stimulation on seizure susceptibility, seizure severity,

seizure latency and seizure duration; (2) a comparison of male and female epileptic chickens in response to intermittent light stimulation, utilizing seizure susceptibility, seizure severity, seizure latency and duration of seizures; (3) the effect of the parentage of epileptic chickens on response to intermittent light stimulation, comparing progeny from matings of epileptic x epileptic, epileptic male x carrier female, carrier male x epileptic female, and carrier x carrier; (4) the effect of prolonged intermittent light stimulation, after a seizure had been induced, on the duration of that seizure and on the incidence and duration of the post seizure depression behavior; (5) the effects of some stress conditions on the response of epileptic chickens to intermittent light stimulation; (6) the determination of the time interval or resting period required by epileptic chickens to undergo another seizure after a complete seizure had been induced.

Part III was a study of segregation of the epi gene and effects of the gene on sex distribution, fertility, embryonic mortality and hatchability.

Part IV was a comparative study of the EEG (electroencephalogram) patterns of epileptic chickens, chickens heterozygous for the epi gene and normal chickens, at rest and during intermittent light stimulation.

2. LITERATURE REVIEW

Epilepsy is an ancient nervous disorder. The concept of seizures and their treatment has been changing continuously due to increasing scientific knowledge of this nervous disorder in humans. Recently, experimental animal models have been used extensively in studying human epilepsy. Experimental animal models which have been used consist of animals with experimentally induced epilepsy and animals with genetically controlled epilepsy. This review of literature will include a general description of human epilepsy and a survey of behavioral and physiological studies of genetically controlled epilepsy in animals. There are many valuable papers in the literature concerning the study in animals with experimentally induced epilepsy; these are not within the scope of the present study and thus are not included in this review.

2.1 Definition.

Epilepsy is a sign of a brain disorder, and is characterized by recurrent sudden paroxysmal disturbances of the neuronal functions of the brain, caused by uncontrollable excessive discharges of the neurons and associated with various clinical symptoms such as complete or partial loss of consciousness, uncontrollable muscular

movements, abnormal sensations and psychic or emotional disturbance (Jasper et al., 1969; Robb, 1965; and others).

2.2 General description of human epilepsy.

2.2.1 Classification of seizures.

In general, epileptic seizures can be classified into four main types according to their behavioral characteristics.

(1) Grand mal seizures (major seizures) are the most severe types of convulsion (Penfield and Erickson, 1941; Putnam, 1945; Schmidt and Wilder, 1968; Scott, 1969; Sutherland and Tait, 1969). They occur with or without warning. The affected individual suddenly loses consciousness, becomes rigid and falls to the ground. Face, trunk and limbs become fixed in irregular postures (tonic contraction). The head and eyes are usually turned to one side, or the affected individual may even turn around several times. After a few seconds, the entire skeletal musculature begins to jerk violently (clonic contraction). The jerking gradually becomes less frequent and less serious until it finally ceases. The individual remains unconscious for a short time, and slowly regains consciousness. He often experiences fatigue, headache, confusion, drowsiness and many other symptoms. Crying and incontinence of urine and feces may occur during convulsions.

Status epilepticus is a term used to describe

severe grand mal seizures occurring in succession, with or without regaining of consciousness between attacks; recovery from status epilepticus is usually incomplete, and death may occasionally occur if no attention is paid to the affected individual during seizures (Putnam, 1945; Robb, 1965; Wishik, 1958).

(2) Petit mal seizures (minor seizures) are characterized by a sudden loss of consciousness with no warning symptoms, usually without muscular movements or with only slight jerking of both arms or blinking of eyes; the affected individual may suddenly return to normal and continue with what he was doing before the attack, and may be unaware of what has happened (Penfield and Erickson, 1941; Putnam, 1945; Schmidt and Wilder, 1968; Scott, 1969; Sutherland and Tait, 1969). Petit mal seizures occur mostly during childhood, beginning after three years of age, and seldom occurring after the onset of puberty (Robb, 1965; Ward et al., 1969).

Petit mal status is a continuation of typical symptoms of petit mal, which is rare but does occur in some instances (Scott, 1969; Tucker and Forster, 1950; Wishik, 1958).

(3) Focal seizures may be motor or sensory. Focal motor seizures (Jacksonian seizures) begin with local uncontrollable jerking in some part of the body, and may gradually extend to involve the whole side of the body,

or may develop into a grand mal seizure (Penfield and Erickson, 1941; Putnam, 1945; Wishik, 1958). Focal sensory seizures begin with local odd or peculiar sensations in the limbs, trunk, or face; the sensations may be visual, olfactory, auditory, gustatory or visceral; the attack may progress into a psychomotor or grand mal seizure (Robb, 1965; Schmidt and Wilder, 1968; Scott, 1969; Ward et al., 1969; Wishik, 1958). The affected individual may or may not lose consciousness during focal seizures (Robb, 1965).

(4) Psychomotor seizures (temporal lobe seizures) are characterized by a sudden performance of some unreasonable or purposeless act such as incontinence, undressing, strange speech, or strange behavior (Horowitz, 1970; Lennox, 1946; Putnam, 1945; Schmidt and Wilder, 1968; Scott, 1969; Ward et al., 1969). The degree of consciousness varies from individual to individual, from slight recollection of what happened during the attack, to recalling nothing at all.

Epilepsy can also be divided into two types, idiopathic and symptomatic (Robb, 1965; Scott, 1969; Ward et al., 1969). Idiopathic epilepsy is the term used to describe seizures in individuals who have no obvious structural abnormality, disease, or any other known abnormality in the brain or in any part of the body. It is generally believed that the disorder is functional or

biochemical in origin. Symptomatic epilepsy is the term used to describe epileptic seizures which have known causes.

2.2.2 Duration of seizures.

The duration of seizures depends upon the type and severity of the disorder. Usually grand mal and petit mal seizures have a short duration (Robb, 1965). The active phase of grand mal seizures may last for one to several minutes (Schmidt and Wilder, 1968; Wishik, 1958). Ward et al. (1969) reported that in 90 per cent of petit mal seizures, the period of unconsciousness ranges from five to 30 seconds. Status epilepticus and petit mal status are prolonged or continuous attacks which may persist for many minutes, hours or days (Kooi, 1971; Putnam, 1945; Robb, 1965; Schmidt and Wilder, 1968; Tucker and Forster, 1950). The duration of focal seizures may be 20 to 30 seconds; spread may occur and produce a grand mal seizure (Schmidt and Wilder, 1968). Psychomotor seizures usually last for a few minutes; in some cases, they are very brief and may be missed by the observer (Lennox, 1946; Schmidt and Wilder, 1968); or they may sometimes last for hours or days (Lennox, 1946).

Robb (1965) classified seizure duration in the following way: (1) very brief, with seizures of about one second; (2) brief, with seizures of about ten seconds; (3) long, with seizures lasting for one minute or more;

and (4) prolonged or continuing, with seizures lasting for an hour or more.

2.2.3. Causes of seizures.

The cause of epilepsy probably involves a number of interacting factors both genetic and environmental.

2.2.3.1 Genetic factors.

In most recent genetic studies, it has been suggested that genetic factors are present in both idiopathic and symptomatic epilepsies (Robb, 1965). It is believed that idiopathic epilepsy has a strong hereditary predisposition (Putnam, 1945; Robb, 1965), but that genetic factors are only partly responsible for symptomatic epilepsy (Penfield and Erickson, 1941; Robb, 1965).

Metrakos and Metrakos (1961, 1961a, 1966, 1969) observed that seizures were more prevalent among near relatives of epileptic individuals than among near relatives of non-epileptics, and that the incidence of seizures among the relatives of affected individuals decreased as the genetic distance increased. In studies of monozygotic and dizygotic twins with epilepsy, EEG abnormalities (Inouye, 1960) and incidence of seizures (Lennox, 1947, 1960) were much higher in monozygotic twins than in dizygotic twins.

The mode of inheritance of different types of

human epilepsy is not clear, but it seems to be controlled by more than one pair of genes, except in centrencephalic epilepsy which is characterized by a bilaterally synchronous three-per-second wave and spike EEG pattern (Metrakos and Metrakos, 1960, 1961, 1961a, 1966, 1969). Metrakos and Metrakos (1961, 1961a) suggested that centrencephalic epilepsy may be controlled by an autosomal dominant gene.

Metrakos and Metrakos (1960) suggested that three groups of genes are responsible for epileptic seizures in man:

(1) threshold genes which are responsible for the susceptibility to seizures;

(2) genes which are capable of producing well-defined forms of epilepsy;

(3) genes producing hereditary cerebral lesions which may be associated with convulsions.

The first group of genes merely control resistance or susceptibility of an individual to convulsive stimulation. The second group of genes may tend to change the basic control organization of an individual and result in certain types of epilepsy. Epileptic seizures are only the secondary or pleiotropic effect of the third group of genes.

2.2.3.2 Non-genetic factors.

Epileptic seizures may be caused by environmental factors such as lesions of the brain (scar formation from

infectious diseases or brain injury, congenital malformation of the brain, local brain atrophy, brain cysts or brain tumors), toxic factors (lead-poisoning, uremic poisoning), local vascular disturbance in the brain, metabolic disturbances (electrolyte and water imbalance, hypoglycemia), nutritional disturbances (vitamin deficiency), emotional disturbances and many others which affect the structure or function of the brain (Penfield and Erickson, 1941; Putnam, 1945; Robb, 1965). The occurrence and severity of seizures produced by these factors depend on the degree of abnormality or disturbance, and the susceptibility or resistance of an individual to seizures (Robb, 1965).

2.2.4 Precipitating factors.

It has long been known that seizures can be induced by auditory, olfactory, tactile, and photic stimulation (Daube, 1965; Robb, 1965; Servit, 1963; Sutherland and Tait, 1969). In some cases, seizures are precipitated by emotional disturbances. Among all these stimuli, photic stimulation is a very effective type and has been used in studies of human epilepsy (Bickford and Klass, 1969; Daube, 1965; Robb, 1965).

2.2.5 Electroencephalographic (EEG) studies of epilepsy.

Following its introduction by Hans Berger in

1929 (Lennox, 1946), the electroencephalographic technique has become a very useful method in research and diagnosis of epilepsy.

In normal individuals, the amplitude and frequency of brain waves are never the same and vary with the conditions under which the brain is functioning. However, the EEG of normal individuals more or less follows certain patterns, the waves becoming slower and less regular during sleep, and faster and higher at times of excitement (Putnam, 1945). In epileptic individuals, there is often some specific deviation from the normal brain wave pattern. Berger in 1932 and 1933 (Hoch and Knight, 1965) observed that seizures were always accompanied by high voltage electrical discharge of the brain, followed by depression and slowing of all brain activity after seizure. The abnormal EEG patterns of grand mal, petit mal and psychomotor seizures have been described by Gibbs and Gibbs (1967), Kooi (1971), Lennox (1946), Putnam (1965), Robb (1965), Rodin and Gonzalez (1966), Schmidt and Wilder (1968), Wishik (1958) and many others. During grand mal seizures, a sequence of spikes increasing in amplitude and frequency can be observed in the tonic phase, and these are replaced by poly spikes interrupted by slow waves when the clonic phase is entered. In petit mal seizures, the EEG pattern is characterized by bilaterally synchronous three-per-second spike and wave activity. In

psychomotor seizures, abnormal spikes, sharp waves, or slow waves with very broad tops localized over the temporal regions of the brain, can always be recorded. The EEG pattern of focal epilepsy is usually characterized by focal spiking, sharp wave activity or rhythmic slow waves (Gibbs and Gibbs, 1967; Kooi, 1971). Kooi (1971) observed that status epilepticus has an EEG pattern of diffuse spiking, and flattening or slowing waves alternate repetitively, usually without any normal activity in between. Petit mal status has a continuous typical three-per-second spike and wave pattern.

An abnormal EEG may occur in epileptic individuals between attacks (Lennox, 1946; Putnam, 1945). Most epileptic individuals show transient disturbances of the EEG by fast, slow or alternately fast and slow waves, depending mainly on the type of seizures an individual usually has. Increasing frequency of occurrence of a series of fast waves or spikes can be recorded many hours, or at least several seconds, prior to a grand mal seizure. The typical spike and wave pattern can be obtained from petit mal patients between attacks.

2.2.6 Mechanisms of epileptic seizures.

About a century ago, Hughlings Jackson (cited by Penfield and Erickson, 1941) hypothesized that focal motor seizures are due to excessive repetitive neuronal

discharges in a certain area of the cerebral cortex; he also suggested that the discharges may spread to neighboring areas of the brain, and may finally involve one whole side of the brain, or may involve the whole brain. The spread may be associated with the march of motor manifestations as shown in the affected individual during seizure. His hypothesis has been confirmed by EEG studies of epilepsy and has become the basic explanation of all types of epileptic seizures (Brain and Walton, 1969; Ward et al., 1969).

Ward et al. (1969) suggested that epilepsy could be caused by individual defective neurons located among normal brain cells. These abnormal neurons may act as pacemakers which serve to trigger more and more cells into abnormal excessive discharges. Epilepsy could also be caused by an abnormal cell environment which affects the normal functions of the nerve cells.

Penfield and Kristiansen (1951) reported that a grand mal seizure is due to intense discharges of neurons originating from the higher brain stem where subcortical motor mechanisms are located, and spreading to all areas of the cortex through neuronal tracts. In petit mal seizures, abnormal discharges are limited to the original ganglionic area. The discharges may reach the higher brain stem through the projection tracts, and may develop into a grand mal seizure if the motor mechanisms in the higher brain stem have received

enough stimulation to discharge in turn.

Robb (1965) reported that the basic disorder of the neuron is due to the instability of electrical potential of the cell membrane caused by imbalance of ionic concentrations on both sides of the cell membrane. Coirault and Jeanneton (1959, as quoted by Sanders et al., 1970), observed that epileptic seizures are generally preceded by retention of intracellular sodium ions. Robb (1965) also suggested that epileptic discharges could be due to defective repolarizing and hyperpolarizing mechanisms.

Sanders et al. (1970) reported that the concentration of ATP (adenosine triphosphate) in the brain consistently decreased prior to the onset of seizures induced by subjecting rats to acute hypoxia, or by treating them with hydroxylamine or pentylenetetrazole (Metrazol). ATP is the energy source used for maintaining the resting membrane potential of the neuron through the function of the sodium-pump, which is the mechanism responsible for sodium extrusion from cells; ATP is also used to support neuronal metabolic activity, to synthesize transmitter substance and to maintain amino-acid metabolism (Schmidt and Wilder, 1968). In their hypoxia experiment, Sanders et al. (1970) were able to extend the latency of seizure of rats by increasing ATP level through increasing oxygen tension, or by injection of sodium succinate.

The brain has been found to be the only tissue containing very high concentrations of glutamic acid, gamma-amino-butyric acid (GABA), and the enzyme glutamic decarboxylase, which converts glutamic acid to GABA (Ochs, 1966; West et al., 1966). This enzyme, together with GABA transaminase, has been found to be very important in supplying essential substrates for ATP production under condition of stress (Sanders, Currie and Woodhall, 1967, as quoted by Sanders et al., 1970). GABA has been suggested as an inhibitory transmitter which acts on the cell membrane and quickly inhibits or depresses its excitability (Curtis, 1969; Krnjevic and Schwartz, 1967; Ochs, 1966; Schmidt and Wilder, 1968; Symonds, 1959). Symonds (1959) suggested that an imbalance of GABA and acetylcholine within the brain could be the cause of epileptic seizures. Wood (1970) observed a decrease in cerebral GABA prior to seizures induced in chicks by hyperbaric oxygen. He found that the critical pressure required for the decrease in cerebral GABA was the same as that required for inducing seizures. Intraperitoneal administration of GABA tended to protect chickens against hyperbaric-oxygen-induced seizures. He suggested that a decrease in cerebral GABA concentration is the primary cause of the seizures.

The decrease in ATP (Sanders et al., 1970) and GABA (Wood, 1970) in the brain prior to the onset of seizures indicate that the metabolism of the glutamate

group of amino acids, which is important in maintaining normal cerebral function, may be impaired. The brain is not able to maintain stable neuronal function, resulting in seizure activity.

Symonds (1959) suggested that an imbalance of GABA and acetylcholine, which is considered to be an excitatory transmitter required for synaptic transmission within the brain could be the cause of the epileptic seizures. Richter and Crossland (1949) found that in rats following an electrogenic seizure, the acetylcholine content of the brain rapidly decreased to half the normal level. Jurgelsky and Thomas (1966) also suggested that seizures may be due to release of excessive acetylcholine in the brain, under the influence of exogenous or endogenous factors.

According to McKhann and Shooter (1969), the abnormalities which are responsible for epileptic seizures, may lie in the structural components of the cell membrane, or in the structural components of the enzymes which maintain the normal biochemical environment in the central nervous system. One of these defects could change the delicate balance of the excitatory and inhibitory mechanisms in the central nervous system. A defective cell membrane may alter the ionic concentration in the cell or at the synaptic site and cause abnormal function of the neuron. A defective enzyme system may result in a

defect or an accumulation of biochemical products which in turn may affect the physiological function of the cell.

2.3 Epilepsy in primates.

Recently, photogenic seizures have been observed in certain species of primates. Killam et al. (1966, 1967, 1967a) found that baboons (Papio papio) obtained from a certain region of Senegal were relatively sensitive to intermittent light stimulation (ILS); 41 to 60 per cent of these animals were found to be photosensitive as compared to the same species of baboon obtained from another region of Senegal (Serbanescu et al., 1968, as quoted by Naquet, 1969) and to other races and species of primates (Killam et al., 1966, 1967b) in which only 10 to 20 per cent of the animals were found to be photosensitive. The frequency of photosensitive epilepsy obtained in any species of primate is much higher than that in humans where the frequency of photosensitive epilepsy is about 1 to 4 per cent (Bickford, 1949; Buchthal and Lennox, 1953; Gastaut et al., 1959; O'Connor, 1964; Trolle, 1953). However, Naquet et al. (1967) tested 38 chimpanzees with ILS and found that none of the animals responded to flickering light.

The effective range of flash frequency of ILS for Papio papio is between 20 and 30 flashes per second

(fps) and the optimum frequency is about 25 fps (Killam et al., 1967; Fischer-Williams et al., 1968; Naquet et al., 1968).

EEG abnormalities and motor manifestions of paroxysm can be observed when the animal responds to ILS (Killam et al., 1966, 1967, 1967a, 1967b; Fischer-Williams et al., 1968; Naquet, 1969; Naquet et al., 1968, 1969). Large amplitude, bilateral and synchronous polyspikes with a frequency close to or independent of the ILS frequency, or polyspike and wave complex at an approximate frequency of 3 per second can be obtained during light stimulation, before the onset of seizures in the affected animals. Motor responses always begin with rapid-clonic jerks of the eyelids for one to several seconds. This may be the only observable sign, or the jerking may progress to facial muscles, the back of the neck, and finally to the whole body. In some cases, general tonic-clonic convulsions may occur. Cries and incontinence of urine often occur during the seizure.

In most animals, abnormal EEG discharges and motor responses cease abruptly at the end of ILS (Naquet, 1969; Naquet et al., 1969). However, in some cases, self-sustained EEG discharges and convulsive movements may continue after the cessation of ILS, but at gradually decreasing frequencies. The after-ILS discharges are generally polyspike and wave complex, they usually last

for 3 to 6 seconds and then stop abruptly. EEG depression may occur before the normal EEG pattern resumes.

Age and sex and the living condition of the animals do not seem to affect the EEG abnormalities and motor manifestions due to ILS (Killam et al., 1967); but the frequency and extent of the paroxysmal discharges are affected by the intensity of light, and increase when the light source is close to the eyes (Naquet, 1969).

2.4 Genetically controlled epilepsy in cattle.

Atkeson et al. (1944) have reported an idiopathic epilepsy found in Brown Swiss cattle. Seizures occur most often when affected animals become excited or are exercised. During a seizure, the affected animal exhibits slight foaming at the mouth, head lowering, tongue chewing and eventually collapses into a coma. Intensity of the seizures varies with individual animals, interval between seizures, and susceptibility to seizures. The first seizures usually occur when the calves are a few months old, and decrease in frequency with increasing age; seizures seldom occur after the animals reach the age of one or two years. Affected animals may permanently lose some control of the hind legs. An autosomal dominant gene is responsible for this affliction.

2.5 Genetically controlled epilepsy in goats.

The first written report of epilepsy in goats was made about 400 B.C. by Hippocrates in which he said ".....Observe the goat, for the animal is most prone to this disease.....Open his head, you will find the brain wet, bathed in a hydroptic fluid of evil odor....." (cited by Penfield and Erickson, 1941). "Stiff legged", "sensitive" or "nervous" goats have been described by Hooper (1916), Lush (1930) and White and Plaskett (1904, as quoted by Lush, 1930). The hind legs are always the first to be affected and become rigid when the animal is frightened by a loud noise. In severe cases, the front legs and the body become involved and the goat may fall to the ground in a rigid condition. The attack lasts for ten to 20 seconds. Recovery always begins from the front end of the body, and moves toward the hind end. A second seizure cannot be elicited for 20 to 30 minutes. The mode of inheritance of this peculiar abnormality is not clear.

2.6 Genetically controlled epilepsy in dogs.

Gene controlled epilepsy has been reported in dogs by Croft (1965), Croft and Stockman (1964), Ebertart (1959), Fox (1965), McGrath (1960), and others. Based on an EEG study of 260 dogs, Croft (1965a, 1968) found that 167 individuals had EEG abnormalities; Poodles, Terriers

and Boxers had the highest percentage affected. Ebertart (1959) suggested that the condition may be related to inbreeding. Genetical analysis by G.E. Mann (cited by Burns and Fraser, 1966) suggested that inherited epilepsy in dogs is due to a single autosomal recessive gene.

The onset of seizures in dogs occurs most frequently between one to three years of age (McGrath, 1960). Epilepsy in Keeshonds may not occur until the dogs reach four years of age (Burns and Fraser, 1966), Croft and Stockman (1964) and Croft (1968) suggested that the affected Keeshonds can be distinguished from normals by EEG at or soon after one year of age.

Grand mal seizures are the most common type occurring in dogs. McGrath (1960) described inherited epilepsy in dogs as follows: "The convulsive seizure described or stimulated is usually of the tonic-clonic variety lasting from 30 seconds to two minutes. The animal with true epilepsy frequently passes urine, feces, or both, during the seizure. Following the attack, the dog recovers a normal state in a very short time in which the animal will appear dazed, thirsty, or hungry". Parry (1949) has given quite a complete description of the clinical manifestions of different types of epileptic seizures in dogs, their precipitating factors and causes of the disease, most of which are quite similar to those occurring in humans.

2.7 Genetically controlled epilepsy in rabbits.

Gene controlled epileptic seizures have been reported in the Vienna White rabbit (Antonitis et al., 1954; Kroll, 1941, as quoted by Gruneberg, 1947; Nachtsheim, 1939, 1940, 1941, as quoted by Gruneberg, 1947), and in Beveren rabbits or their crosses which have a physical appearance similar to Nachtsheim's Vienna White rabbits (Hohenboken and Nellhaus, 1970; Nellhaus, 1958, 1963).

The pattern of seizures is in many ways similar to human epilepsy and to the seizures described in the mouse and rat. It consists of a running phase, a convulsive phase, and a recovering phase. At the beginning, a perfectly normal animal suddenly becomes agitated, runs and leaps wildly about the cage, drops down and loses consciousness. The convulsive phase then begins; the head and front part of the body first become rigid, it rapidly involves the hind part of the body, until the whole body becomes rigid, and the hind legs usually stretch backwards. Vigorous clonic muscular contractions then begin, involving mainly the fore and hind legs of the animal. Cries, lip biting, salivation, and incontinence of urine and feces frequently occur during the seizure. The animal then begins to recover; the front legs are usually paralysed at the beginning of recovery, and finally become completely normal.

Occasionally, incomplete or aborted seizures may occur in these rabbits (Gruneberg, 1947; Nellhaus, 1963). They consist only of the wild running phase without loss of consciousness and tonic-clonic convulsions, or with a slight tonic convulsion. Status epilepticus had also been observed by Nellhaus (1963), especially in young rabbits. Death may result immediately after the seizure.

Seizures occur mostly when the animal is disturbed such as during feeding or handling, or when the animal is subjected to auditory stimulation (Nachtsheim, 1939, 1942, as quoted by Hohenboken and Nellhaus, 1970; Nellhaus, 1963).

The latency and duration of different phases of the seizure are highly variable among animals, and also in the same animal tested from time to time (Antonitis et al., 1954; Nellhaus, 1963), especially in strains with a high susceptibility to audiogenic seizures (Antonitis et al., 1954). Nellhaus (1963) reported that the latency of audiogenic seizures in these rabbits varied from two to 45 seconds; in some less sensitive animals, the latency went up to almost 90 seconds. The tonic and clonic convulsions each last between ten to 20 seconds. The animal may recover immediately after the seizure, or may take up to half an hour to recover completely. Antonitis et al. (1954) found that the latency was much shorter and

the durations of convulsive and recovering phases were much longer in the achondroplastic-epileptic (AcEp) strain of rabbits which were selected for high susceptibility to audiogenic seizures as compared to the achondroplastic (Ac) strain which served as a control. The average latency of 12 animals from the AcEp strain was 6.8 seconds and the average latency of five animals from the Ac strain was 17 seconds. The duration of seizure and recovery periods were 73.9 and 213.9 seconds in the AcEp strain, and 22.6 and 39.4 seconds in the Ac strain, respectively. In the unselected rabbits studied by Nachtsheim (1939, 1940, 1941, as quoted by Gruneberg, 1947) the average seizure duration was about 25 seconds and the animals rarely had a seizure lasting up to 60 seconds. Status epilepticus described by Nellhaus (1963) lasted up to 30 minutes.

Frequency of seizures was found to be high in the AcEp strain (33.3%) as compared to 16.2% in the Ac strain (Antonitis et al., 1954). Nellhaus (1958, 1963) also found a significant increase in seizure susceptibility in animals obtained through continuous inbreeding of highly susceptible animals. After a few generations of inbreeding, 100 per cent susceptible animals could be obtained in some litters; whereas susceptibility in non-selected groups was only about ten to 11 per cent (Hohenboken and Nellhaus, 1970; Nellhaus, 1963).

Seizures occurred more frequently during summer than during winter (Nachtsheim, 1939, 1940, 1941, as quoted by Gruneberg, 1947). No seizure appeared in rabbits during the first three weeks after birth. The first attack occurred mostly between two and six months of age, with the peak of seizure susceptibility at the seventh and eighth week (Hohenboken and Nellhaus, 1970; Nellhaus, 1963). The frequency and severity of seizures tended to decrease with increasing age, and seldom occurred in animals after 18 months of age. Seizures occurred equally in both sexes (Nellhaus, 1963).

Nachtsheim (1939, 1940, 1941, as quoted by Gruneberg, 1947) found that epilepsy in rabbits was always associated with the Vienna White factor which causes the white coat and blue eyes of the breed. The author could not separate epilepsy from the Vienna White factor through breeding. The expressivity of the gene was incomplete, being about 75 per cent. He suggested that the expressivity of the gene was more likely to be suppressed by the existence of modifying genes. Antonitis et al. (1954) confirmed that the susceptibility to seizures was controlled by an autosomal recessive gene with incomplete penetrance.

In EEG studies, spontaneous spikes have been recorded in highly seizure-susceptible rabbits (Nellhaus, 1963). Some spike and wave discharges also appear in the

EEG of susceptible animals during auditory stimulation. No visible histological abnormality could be found in the brains of affected animals.

2.8 Genetically controlled epilepsy in mice.

Inherited epilepsy has been reported in some species of deer mice, Peromyscus (Chance and Yaxley, 1950; Dice, 1935; Summer, 1932; Watson, 1939, as quoted by Gruneberg, 1947), and in many strains of house mice, Mus musculus (Frings et al., 1951; Fuller, 1950; Fuller et al., 1950; Fuller and Rappaport, 1952; Fuller and Sjursen, 1967; Ginsburg and Hovda, 1947; Ginsburg and Huth, 1947; Ginsburg et al., 1950; Hall, 1947; Mirsky et al., 1943; Vicari, 1947, 1948, 1948a, 1950; Witt and Hall, 1949). There are similarities of seizure pattern in deer mice and house mice (Frings et al., 1951), and the seizures are similar to those described in rabbits (Gruneberg, 1947).

2.8.1 Seizures in deer mice.

Seizures can be induced by auditory stimulation in many species of deer mice as reported by Dice (1935), Summer (1932) and Watson (1939, as quoted by Gruneberg, 1947), and by olfactory stimulation in one particular species, Peromyscus rufinus, as reported by Gruneberg (1947).

The audiogenic seizures usually begin with a startle reaction, preceded by a crouching response (Chance and Yaxley, 1950; Dice, 1935; Gruneberg, 1947), and then the mouse suddenly dashes about the cage for about 20 seconds. It falls over on its side and begins to convulse. The convulsive phase, different from that in rabbits, begins with a clonic convulsion followed by tonic convulsive movements. It takes a minute or two for the animal to recover from the seizure. However, in some cases, the recovery period may last up to 25 minutes. Death may occur during the tonic convulsion. The latency and duration of running, convulsive and recovering phases vary from animal to animal (Dice, 1935).

Seizures may be less severe and consist only of the running response, without clonic-tonic convulsions, or they may look like a kind of stupor, in which the animal looks and acts as if it is recovering from a complete seizure, or semi-stupor in which the animal acts like it is in a stupor but is capable of moving about (Frings et al., 1951; Gruneberg, 1947). A bouncing seizure also has been observed in which the animal bounces with its hind legs for some time and eventually walks away.

In some strains of deer mice, seizures are associated with waltzing behavior in which the epileptic seizure is always preceded by a few seconds of rapid

waltzing (Chance and Yaxley, 1950; Watson, 1939, as quoted by Gruneberg, 1947).

There is no difference between sexes in audiogenic seizures of these animals, but the severity of seizure tends to decrease with age and it requires greater and greater stimulation to provoke a seizure, until it eventually disappears in old animals which mostly become deaf (Dice, 1935; Watson, 1939, as quoted by Gruneberg, 1947). The animals become deaf at a mean age of 6.8 $\pm$ 0.5 months.

The inheritance of audiogenic seizures is not clear in other species of deer mice, but in Peromyscus maniculatus artemisiae, Dice (1935) and Watson (1939, as quoted by Gruneberg, 1947) suggest that the character is controlled by an autosomal recessive gene.

The epileptic seizures in Peromyscus rufinus have been found to be induced only by olfactory stimulation, such as by tobacco smoke, oily rags, or incense (Gruneberg, 1947). When the stimulus is applied to the affected animal, it stands on its hind legs for a while and gradually falls over on its back, this behavior being repeated several times and then is followed by violent bouncing for a period of 10 to 15 seconds. The affected animal responds to olfactory stimulation throughout its life. The condition is probably controlled by two non-allelic autosomal recessive genes.

2.8.2 Seizures in house mice.

Frings et al. (1951) have found that there are similarities in the seizure pattern of deer mice and house mice. However, there are some differences between them. In the running phase, the house mouse does not run uncoordinatedly as does the deer mouse; no bouncing seizures are observed in the house mouse. In highly susceptible animals, death commonly occurs immediately after the seizure in the house mouse, but only occasionally in the deer mouse.

Incomplete seizures also have been found in the house mouse. They vary from merely a wild running activity (Frings et al., 1951; Witt and Hall, 1949) to an act of rolling in an uncoordinated pattern following the running activity (Frings et al., 1951).

Seizures in the house mouse are mostly induced by auditory stimulation (Frings et al., 1951; Fuller et al., 1950; Fuller and Sjursen, 1967; Ginsburg and Hovda, 1947; Hall, 1947; Witt and Hall, 1949; and others), but can also be induced by electric shock (Stone et al., 1949).

Audiogenic seizures vary considerably between strains (Frings et al., 1951; Fuller et al., 1950; Fuller and Sjursen, 1967; Ginsburg and Hovda, 1947; Hall, 1947; Vicari, 1947, 1948; Witt and Hall, 1949), and between individuals within the same strain, or even within the same litter (Frings et al., 1951; Fuller et al., 1950).

Witt and Hall (1949) found that the average latency of seizures in mice, including both highly susceptible and highly resistant strains, was about 30 seconds with a range of three to 105 seconds. Frings et al. (1951) found a similar result in three strains of mice which varied in susceptibility to seizures; the average latency in these strains was about 30 to 35 seconds; but the differences among these strains were not significant. However, Frings and Frings (1952, 1953) found that the latency of seizures could be modified through selective breeding; the latency in highly susceptible strains of mice was about six seconds, as compared to the latency of the original unselected mice which was about 27 seconds. By crossing highly susceptible and highly resistant strains, Fuller et al. (1950) concluded that the highly susceptible parent was the one which contributed genes reducing latency and the highly resistant parent was the one contributing genes increasing latency.

Fuller and Smith (1953) and Fuller and Sjursen (1967) found that in many mice, there were often two running activities which were separated by a period of quiescence. These occurred before the onset of clonic-tonic convulsive movements and produced a bimodal distribution of the time of onset of the convulsive phase. Fuller and Sjursen (1967) found that the first wave of

convulsions occurred mainly within 20 seconds and the second wave of convulsions occurred approximately 36 to 56 seconds after the beginning of auditory stimulation. The clonic convulsive phase was about one second or more which was usually shorter than the following tonic convulsive phase which might last for about ten seconds (Frings et al., 1951).

Seizures often ended with death in the house mouse; males seemed to be more susceptible to death in seizures than females (Frings et al., 1951). However, some mice recovered from the seizure, the average recovering time reported by Frings et al. (1951) being 30 to 50 seconds with a range of ten to 200 seconds. They also found that recovery time depended on the severity of the seizure. Fuller (1950), Fuller et al. (1950), and Fuller and Sjursen (1967) observed that the ability to recover from seizures was inherited independently of seizure susceptibility.

Witt and Hall (1949) reported that the first occurrence of seizures in mice is at about 30 days of age. Vicari (1950) found that the age of onset of the most severe type of seizures, which usually resulted in death of the affected animals, was between 20 and 80 days of age, with the peak at about 29 to 33 days; 92 per cent of the animals had seizures in these ages and 70 per cent of them died after the seizure. The age of onset of less

severe seizures, which consisted of only clonic-tonic convulsions but no death of the affected animals, was between 60 to 210 days with a peak at 90 days of age. Swinyard et al. (1963) reported that the first seizure in mice could be induced as early as ten days of age and which reached a maximum incidence and severity at about 22 days, after which the incidence of seizures decreased with increasing age and weight of the animals. Ginsburg and Huth (1947) and Fuller and Sjursen (1967) also found that in most susceptible animals, the susceptibility to seizures decreased with age. Witt and Hall (1949) and Fuller and Sjursen (1967) found that seizure mechanism was independent of the body weight.

Frings and Frings (1953) found that the degree of susceptibility to seizures, the severity of seizures, and the age of onset of seizures can be changed through selection. They produced four strains of mice. Two of these strains had 90 to 100 per cent seizure susceptibility when tested between 15 and 50 days of age; one of these highly susceptible strains of mice produced seizures consisting of severe clonic-tonic convulsions and the other strain of mice produced seizures consisting of clonic convulsions only; in both strains of animals death seldom followed a seizure. The third strain of mice had a very low susceptibility to seizures; zero to ten per cent exhibited seizure susceptibility when tested between 15

and 50 days of age. Seventy per cent of the animals did not respond to auditory stimulation. The fourth strain was selected for seizure incidence at a certain age; animals of this strain had seizures regularly between 17 to 27 days of age but not after that. They concluded that the susceptibility to and severity of seizures, age distribution of seizures, and the resistance to death during seizures was inherited independently.

Fuller and Rappaport (1952) found that cooling the animals in cold water, thereby reducing the body temperature protected against audiogenic seizures; the lower the water temperature, the higher was the protective effect. Later Essman and Sudak (1964) confirmed that the protective effect was due to reduction of body temperature. They found that by subjecting the animals to cold air ($2 \pm 0.5°C$) or to cold detergent, the latency of seizures was increased in 92 per cent of the animals when the colonic temperature of the mouse was reduced from $37.2 \pm 0.6°C$ (normal temperature) to between 34.5° and 29°C; further decrease in the colonic temperature to between 29° and 27°C resulted in protecting 69 per cent of the animals completely against seizures caused by auditory stimulation; no animal was found to respond to sound when the colonic temperature was lower than 27°C. However, the cold effect was only temporary, and the protective effect decreased with gradual increase of colonic temperature in the treated

animals.

The inheritance of audiogenic seizures in house mice is controversial. In the early reports of Witt and Hall (1949) and Ginsburg et al. (1950), a single autosomal dominant gene was suggested as being essential for controlling seizure susceptibility, but expression of the trait was thought to be influenced by one or more modifying genes. Later, Ginsburg and Starbuck-Miller (1963, as quoted by Fuller and Sjursen, 1967) indicated that two dominant genes were involved. Collins and Fuller (1968) suggested that if seizure susceptibility was determined in a single test, inheritance could be explained by the action of an autosomal recessive gene; but if multiple tests were used to determine seizure susceptibility of mice, the genetic interpretation became complex and required the action of more than one pair of codominant genes. The work of Frings and Frings (1953), Fuller et al. (1950) and Fuller and Sjursen (1967), favoured multigenic inheritance; they found that mice of low susceptibility to seizures could produce highly susceptible offspring and vice versa.

Epileptic seizures in mice can also be induced by means of electric shock. Stone et al. (1949) found that electrogenic seizures were very similar to those induced by auditory stimulation. No difference was found between sexes, but resistance to death following seizures was

found to differ among strains. In an albino strain, 83.9 per cent death followed 30 seizures; in "extreme dilute" mice, only 11 per cent death followed 27 seizures; and in a "fawn" strain, 25 per cent death followed 40 seizures.

2.9 Genetically controlled epilepsy in rats.

Audiogenic seizures were first reported in rats by Maier (1939) and were extensively studied by him and many others (Bayroff, 1940; Bernhardt et al., 1941; Martin and Hall, 1941; Morgan and Galambos, 1942; Morgan and Morgan, 1939; and others).

Rats are generally more susceptible to seizures than mice. Auer and Smith (1940) reported that approximately 30 per cent of over 400 rats responded to auditory stimulation and repeatedly underwent seizures, while Mirsky et al. (1943) found only five per cent of 500 mice reacted to auditory stimulation.

In general, the seizure pattern in rats is similar to that of mice except that in rats the tonic convulsion, as in rabbits, precedes the clonic convulsion, and the seizure seldom ends with death of the animal (Auer and Smith, 1940; Finger, 1942; Mirsky et al., 1943; Patton, 1941; Patton and Karn, 1941; Smith, 1941). During the tonic immobility stage, the rat usually can be moulded into various positions, but this cannot be done in

the mouse (Mirsky et al., 1943; Patton and Karn, 1941). Gruneberg (1947) reported that individual seizures were variable and might be abortive.

Finger (1947) reported that the latency of audiogenic seizures in rats varied from less than five seconds to somewhat more than a minute. However, it might vary among strains; by using the same source of auditory stimulus (Galton whistle), Smith (1941) found that the average latency of seizures of white rats was 11.2 seconds, with approximately half the cases having a latency of one to seven seconds; but Morgan (1941) reported that in his brown rats the latency was 40 to 60 seconds, which was much longer than that found by Smith (1941). Morgan (1941) also found that the latency of seizures in rats was generally rather stable from seizure to seizure, and was not affected by age, sex, seizure susceptibility of the animals, the intensity and frequency of the auditory stimulation, or the type of test-condition; but it tended to be shortened as more and more seizures were produced in the same rats. Finger (1943) also found no evidence that age systematically influenced the latency or the severity of seizures.

As in the house mouse, seizures in rats might begin with one or two running activities preceding severe muscular contractions (Auer and Smith, 1940; Smith, 1941). The first running phase lasted only a few seconds. The

second running phase and the quiescence period which lay between the first and second running phases, lasted for about 20 seconds each. The convulsive phase consisted of tonic convulsions which lasted for five to 43 seconds, followed by clonic convulsions which lasted from 70 to 330 seconds. The entire seizure might last up to three and one half minutes.

The susceptibility to audiogenic seizures in rats is affected by the physiological condition of the animals, which is correlated with age, sex, body temperature and physical situation (e.g. fatigue), and by administration of drugs or vitamins which will modify the physiological condition of the animals.

Finger (1943) and Gruneberg (1947) found that seizure susceptibility was low in very young animals, increased rapidly after weaning, and gradually decreased in older animals. Maier and Glaser (1942a) found that susceptibility to seizures in rats reached the maximum level at about 20 weeks of age, and changed little between 20 and 40 weeks of age.

Maier and Glaser (1942a) did not find sex to be related to seizure susceptibility of rats. However, Farris and Yeakel (1942, 1942a) reported that sex differences seemed to exist in the strain of rats which they studied; males reacted more frequently than females at 26 to 40 days of age; after 125 days of age, the

frequency of seizures in males was sharply reduced; by 240 days of age the frequency of response in females was found to be about twice that of the males; however, in advanced age, susceptibility to seizures was found to decrease considerably in both sexes.

Maier and Glaser (1942) found that fatigue or lowering the body temperature of the rats could prevent seizures. They found that rats subjected to 45 minutes of swimming in water preceding auditory stimulation were more resistant to the stimulus than rats with a two-minute period of swimming. Water at low temperature was more effective than water at high temperature. They suggested that the reduction of body temperature and fatigue due to swimming decreased the available energy of the rats and were therefore responsible for the temporary disability of the convulsive response of the animals. Humphrey and Marcuse (1941) also found that exercise during or prior to auditory stimulation markedly reduced the frequency of seizures.

Maier et al. (1941) and Sacks and Maier (1942) found that a subconvulsive dose of Metrazol could temporarily cause rats which did not respond to auditory stimulation to show seizures. Humphrey (1941) was able to reduce the frequency of seizures in rats by using atropine (a parasympathetic depressant) and to increase the frequency of seizures by using nicotine (a ganglionic

excitant).

Patton (1941) and Patton et al. (1941, 1942, 1942a) found that the frequency of seizures decreased in rats which were fed a diet supplied with additional vitamin B complex, including vitamins B1, B2, and B6.

The susceptibility to audiogenic seizures in rats is also affected by the emotional or psychological state of the animals. Maier (1939), Maier and Glaser (1940), and Maier and Klee (1941) found that rats in a conflict situation were more susceptible to auditory stimulation, and adaptation to auditory stimulation took place relatively slower in these animals than in rats which were not in the conflict situation. Humphrey and Marcuse (1939) also found that seizure susceptibility in rats was increased in conflict situations. However, Morgan and Waldman (1941) found that a conflict situation did not help to induce seizures in rats which failed to respond to auditory stimulation previously; and the conflict situation did not increase the number of seizures in five susceptible rats which were tested repeatedly up to fifty trials; they obtained 14 seizures out of 25 tests under a normal testing condition, and 15 seizures out of 25 tests under the conflict situation.

Humphrey and Marcuse (1939) found that rats were more susceptible to audiogenic seizures during an emotional condition which was created by swinging the

animals in a cage during auditory stimulation. Humphrey and Marcuse (1941) found that the frequency of audiogenic seizures in rats was greatly decreased by taming through careful handling for a certain period of time each day. Sixty-two per cent of the 74 untamed rats had seizures as compared to eight per cent of the 74 tamed rats tested by auditory stimulation in the first two days after the taming process; during a continuous period of 25 days after that, frequency of seizures in untamed rats was much higher than in tamed rats.

Patton and Karn (1941) found that daily auditory stimulation did not influence the seizure susceptibility in rats. However, Maier and Glaser (1942, 1942a) found that repeated testing produced a lowered susceptibility in rats. They suggested that the reduction in seizure susceptibility might be due to adjustments made by the animal; but the adjustments did not last indefinitely. They disappeared after the auditory stimulation had been withheld for a period of time.

Maier and Glaser (1942) found that susceptibility to audiogenic seizures in rats could be affected by exposing the animals to sound stimulus insufficient to cause seizures. The frequency of seizures was greatly reduced, and some of the highly susceptible animals were prevented from producing seizures under an effective sound stimulus after previously being subjected to an ineffective

sound stimulus. They also found that rats which did not produce seizures in a large enclosure could be induced to undergo seizures by placing them in a smaller enclosure in which their movements were greatly restricted.

The susceptibility to audiogenic seizures in rats is also affected by the frequency and intensity of the auditory stimuli. By using a pure tone produced from an electric oscillator, Gould and Morgan (1941, 1942) found that the sensitivity of rats to auditory stimulation increased greatly with the increase of stimulating frequency. Morgan and Gould (1941) found that a frequency of 20 kc (kilocycles) per second was relatively more effective in inducing seizures in rats than a frequency of 12 or 16 kc per second, and no seizures could be obtained by using auditory stimuli of a frequency lower than 12 kc per second. Gould and Morgan (1942) found that the sensitivity of rats seemed to increase continuously with an increase in frequency of auditory stimulus up to 40 kc per second.

Morgan and Waldman (1941) found that in rats an increase in the intensity of an auditory stimulus was followed by an increase in incidence of seizures. Morgan and Galambos (1942) found that the intensity required to maintain a seizure after it had been induced was less than the intensity required to initiate a seizure; however, greater and greater intensity was

required to maintain the seizure which proceeded with time, and the animal eventually went into a coma.

Lindsley et al. (1942) studied the brain waves and heart rate in rats subjected to auditory stimulation. They found that various phases of a seizure were associated with abnormalities of EEG. In non-seizure recordings, the changes of EEG and heart rate were not marked. Heart rate increased and/or decreased prior to the seizure; they explained that this was due to the dual action of the autonomic system preceding the seizure, in which sympathetic and parasympathetic systems had been involved, but the former tended to predominate.

Audiogenic seizures in rats were first thought to be inherited as an autosomal dominant trait by Maier and Glaser (1940) but subsequent reports by Finger (1943), Griffiths (1942), and Maier (1943) indicated a multigenic inheritance. Most investigators agree with the latter view (Fuller and Thompson, 1960). Gruneberg (1947) reported that none of the strains that had been tested was completely resistant to auditory stimulation. Various strains were found to differ in their susceptibility to seizures (Farris and Yeakel, 1943; Maier, 1943; Maier and Glaser, 1940). Maier (1943) found that his high seizure-susceptible (unstable) strain not only responded more frequently to auditory stimulation, but also tended to transmit the defect more readily than did the low seizure-

susceptible (stable) strain; he suggested that the strain difference in transmission could be due to the different aggregation of genes which were responsible for seizures. He also reported that strains with high or low susceptibility to seizures could be produced through selective breeding, and that selection was much more effective in the low seizure-susceptible (stable) strain than in the high seizure-susceptible (unstable) strain.

2.10 Abnormalities of the nervous system in poultry.

Several abnormalities of the nervous system have been reported in poultry. These are congenital loco in chickens (Knowlton, 1929, as quoted by Hutt, 1949), in turkeys (Cole, 1957), and in Japanese quail (Sittmann et al., 1965); star-gazing in Japanese quail (Savage and Collins, 1972); congenital tremor in chickens (Hutt and Child, 1934) and in ducks (Dyrendahl, 1958); shaker (Scott et al., 1950) and jittery (Bohren, 1950; Godfrey et al., 1953) in chickens; vibrator in turkeys (Coleman et al., 1960; Kulenkamp et al., 1968); paroxysm in chickens (Cole, 1961); and epileptiform seizures in chickens (Crawford, 1969, 1970).

Congenital loco (lo) is a nervous system disorder found in chickens (Knowlton, 1929, as quoted by Hutt, 1949), turkeys (Cole, 1957) and Japanese quail (Sittmann et al., 1965). The disorder is caused by an

autosomal recessive gene. The symptoms are similar in all the three species but vary in the degree of severity. The affected individual is unable to stand longer than a few seconds, the head is drawn backwards and upwards over the back or to either side of the body, and the individual topples over on its back or side; the performance is quickly repeated after the individual regains an upright position.

The symptoms appear as soon as the affected individuals are hatched (Knowlton, 1929, as quoted by Hutt, 1949; Cole, 1957; Sittmann et al., 1965). Affected individuals have great difficulty in obtaining food and water, thus mortality is high in the first week of age. Cole (1957) found that the disorder in poults was more severe than in day-old chicks. In affected poults (Cole, 1957) and in baby quail (Sittmann et al., 1965), the symptoms were found to be intensified when the affected individuals were excited. Sittmann et al. (1965) found that the severity of disorder gradually decreased in loco quail during the first few days of life until the symptoms were merely perceptible at about one week of age even when they were excited; after that the affected birds began to respond to excitement by rapid circling in a small area for a short time and then they rapidly returned to normal again.

All of the affected chickens studied by

Knowlton (1929, as quoted by Hutt, 1949) died within nine days after hatching. The affected poults studied by Cole (1957) were unable to live longer than a week even when they were artificially fed. The disorder was also highly lethal in Japanese quail but a few females were successfully reared to sexual maturity and were able to produce offspring (Sittmann et al., 1965).

No gross or microscopic lesions were found in the brains of affected turkeys (Cole, 1957) or affected quail (Sittmann et al., 1965).

Savage and Collins (1972) reported "star-gazing" (sg) in Japanese quail, an inherited nervous disorder caused by an autosomal recessive gene with a high degree (98.6 per cent) of penetrance. The affected individuals had symptoms similar to those of loco chicks, quail and poults, but they grew at a normal rate and the star-gazing symptoms were detected only occasionally in quail between hatching and three weeks of age, and tended to become more pronounced in older birds. The affected birds could be distinguished from the normals by suddenly confining them in a small enclosure or by placing an opaque object above them. Under these conditions the affected individuals responded immediately by drawing their heads backwards; the affected individuals might remain motionless, they might walk rapidly backwards or rotate (usually counter clockwise) in this position. Occasionally the

affected birds might squat on their hocks with the head held backwards or turned to one side of the body. The symptoms might last only for a few seconds or for several minutes.

Congenital tremor has been described in chickens by Hutt and Child (1934) and in ducks by Dyrendahl (1958). The affected individuals show various degrees of tremor which involve the whole body; the body balance is poor; the trembling movements are constant while the affected individuals are standing, but cease when they lie down.

In chickens, Hutt and Child (1934) found that 88 per cent of the affected chicks died within a month after hatching, and only one out of 35 affected birds survived to sexual maturity. Among those surviving longer than one week the degree of tremor gradually decreased and became less and less obvious until no sign of the tremor was evident after eight weeks of age.

Dyrendahl (1958) found that in ducks most of the affected ducklings died within three days of age, but a few were able to live to sexual maturity and produced offspring. One drake and one duck were studied by the author. He found that the male was more severely affected than the female. The male was unable to swim because of difficulty in maintaining balance of the body and the tremor condition became more severe in water. The tremor

mainly involved the head and neck regions of the female; she was able to balance herself and was able to swim normally in water. No lesion of the brain was found in the affected ducklings (Dyrendahl, 1958).

Hutt and Child (1934) reported that in chickens, the tremor condition was not induced by excitement or by sudden shock; but Dyrendahl (1958) reported that in ducks, the condition was much more severe when the affected birds were frightened or excited.

The tremor condition was found to be hereditary in chickens, but the mode of inheritance is not clear (Hutt and Child, 1934); in ducks, the condition was caused by an autosomal recessive gene (Dyrendahl, 1958).

"Shaker" is another nervous disorder in chickens reported by Scott et al. (1950). The abnormality could not be detected until the affected chicks reached about 18 days of age. In the affected birds, the head and neck shook rapidly, and the condition became more and more severe with increasing age until by ten weeks of age most of the birds had great difficulty in walking; after 14 weeks of age, most of the birds were unable to stand because of the severe shaking of the head. Excitement was found to intensify the symptoms. Most of the affected birds died from malnutrition but a few females survived to sexual maturity. Scott et al. (1950) found that almost a complete degeneration of the Purkinje cells occurred in

the cerebellar area of the brain in affected birds. The condition was caused by a sex-linked recessive gene.

"Jittery", a condition with symptoms similar to those of "shaker" was reported in chickens by Bohren (1950) and Godfrey et al. (1953). The main difference between "shaker" and "jittery" is in the time of onset of the symptoms; the "jittery" condition appears in affected chicks immediately after hatching, whereas "shaker" chicks cannot be identified until they are 18 days old. In "jittery" chicks, the head shakes rapidly and is retracted over the back. Godfrey et al. (1953) reported that 75 per cent of the "jittery" chicks died in the first two days after hatching and mortality increased to about 90 per cent at the end of the first week. However, about one to two per cent of the affected birds, all of them females, survived to sexual maturity. In addition to those symptoms described in the chicks, the adult hens circled rapidly when frightened. As in "shaker", the "jittery" chicks also showed a marked degeneration of the Purkinje cells of the cerebellar region (Godfrey et al., 1953). A sex-linked recessive gene was responsible for the "jittery" condition in chickens (Bohren, 1950; Godfrey et al., 1953).

In turkeys, a sex-linked recessive mutation called "vibrator" (vi) has been reported by Coleman et al. (1960) and Kulenkamp et al. (1968). Like "shaker" and

"jittery" in chickens, affected turkey poults show a very rapid shaking of the head and neck region, but the symptoms in turkeys decrease with age; in adult turkeys, the vibration can be detected only by lightly grasping the head or neck region of the affected birds. Kulenkamp et al. (1968) found that the mutant gene had no adverse effect on the fertility, hatchability, or growth rate of turkeys, except that mortality was higher in affected birds than in normals.

Paroxysm (px) is another recessive sex-linked lethal reported by Cole (1961) in chickens. The first attack occurs between 12 days and six weeks of age; the affected chick, when frightened by auditory or visual stimulation, or any disturbance which produces a startle effect, begins to move rapidly across the floor and falls with the head turning over the back and the wings beating violently. The legs become rigid and extend backwards, with the whole body in a tetanic and trembling condition for about ten seconds. Then the bird relaxes and rests quietly for varying lengths of time, depending upon the degree of stimulation. Finally it recovers. The next attack cannot be induced for at least 30 minutes after the first. After several attacks, the birds show a syndrome of poor growth, spread tail, and stilted gait. All the affected birds die by 14 weeks of age. The lethal gene has no adverse effect on hatchability or early growth.

No structural abnormality or microscopic lesion has been found in the brains of affected birds.

Epileptiform seizures in the Fayoumi breed of chickens have been reported by Crawford (1969, 1970). The symptoms mentioned in the introduction to this thesis are in some respects similar to those of sex-linked paroxysm described by Cole (1961). Crawford (1969, 1970) reported that epileptiform seizures occur in day-old chicks, and recur in birds throughout the brooding and rearing periods and adulthood. Individual seizures vary in duration and severity. Seizures can be induced by various factors such as strong visual and auditory stimuli and by muscular fatigue. Growth rate, livability and fertility are not affected by the mutant gene. Linkage studies showed that the epileptic gene is independent of dominant white (I), naked neck (Na), polydactyly (Po), frizzle (F), rose comb (R), pea comb (P), and crest (Cr). The symbol epi was suggested for this autosomal recessive mutant.

3. MATERIALS AND METHODS

3.1 Breeding stock.

The birds used in the present studies were derived from a population of 11 epileptic males (epi epi), 27 epileptic females (epi epi), 3 epilepsy carrier males (Epi epi), and 20 epilepsy carrier females (Epi epi). These original birds will be referred to as P_1 (first parental generation) in the following paragraphs.

The P_1 generation used in these studies was the fifth generation of the epilepsy population following discovery in 1963 of the causative mutant gene in the Fayoumi breed (Crawford, 1972). In establishing a population which carried the new mutant, affected Fayoumis had been crossed with the Ottawa Meat Control Strain and with the Kentville White Leghorn Control Strain. In the second generation, the University of Saskatchewan strain of White Leghorns had been added along with a bantam naked neck stock. These four strains had been introduced to facilitate linkage studies. In subsequent generations all five of the orginal stocks were represented in the population and were interbred.

Two hundred and sixty-one birds were obtained from various matings of P_1 birds for use as the second parental generation (P_2). They consisted of birds from matings of epileptic x epileptic, epileptic x carrier,

and carrier x carrier. Numbers of birds, their parentage, and hatching dates were as follows:

Hatching date	No. of birds	Parentage: E x E	E x C	C x C
June 24, 1969	103	50	34	19
July 22, 1969	133	87	33	13
July 24, 1969	25	15	8	2
	261	152	75	34

The genotypes of these birds (P_2) were determined by backcrossing them to the parental stock (P_1) from January to March of 1970; 29 epileptic males, 30 epileptic females, 26 epilepsy carrier males, 28 epilepsy carrier females, three normal males and two normal females were identified and were used to produce birds with known genotype for experimental work and for production of P_3.

P_3 consisted of 25 epileptic males, 30 epileptic females, 16 epilepsy carrier males, 14 epilepsy carrier females, nine normal males and seven normal females. They were obtained from several hatches; their hatching dates and number of birds obtained from each hatch were as follows:

Hatching date	No. of birds	Genotype		
		Epileptic (*epi epi*)	Carrier (*Epi epi*)	Normal (*Epi Epi*)
Feb. 14, 1970	12	9	3	0
Mar. 1, 1970	20	19	1	0
Apr. 21, 1970	12	2	10	0
Apr. 27, 1970	10	2	4	4
June 22, 1970	10	2	2	6
Sept. 3, 1970	13	8	2	3
Sept. 12, 1970	15	10	3	2
Sept. 28, 1970	10	4	5	1
	102	56	30	16

3.2 Experimental stock.

Birds used as experimental stock in these studies were produced by P_1, P_2, and P_3 from 1969 to 1971. The numbers of birds used in each experiment, their parentage and hatching dates are shown in Table 1; it should be noted that some individuals were used in more than one experiment. A total of 2025 birds were used in 12 experiments; these consisted of 1603 epileptic (*epi epi*), 120 epilepsy carrier (*Epi epi*), 105 normal (*Epi Epi*), and 197 non-epileptic birds with unknown genotype (*Epi*-). The epileptic birds were obtained from matings of epileptic x epileptic, epileptic x carrier, and carrier x carrier. Epileptic birds which were obtained

from matings of epileptic x carrier and carrier x carrier were identified after observing at least one spontaneous seizure, or a seizure induced by auditory or intermittent light stimulation. Epilepsy carriers were produced from matings of normal x epileptic. Normal birds were obtained from matings of normal x normal. Normal birds of unknown genotype (Epi-) were produced from matings of carrier x carrier; these birds did not show any spontaneous seizures, audiogenic seizures or photogenic seizures at day-old or thereafter, and thus were assumed to be either homozygous normal or epilepsy carrier (heterozygous).

3.3 Management.

At hatching, chicks were individually marked with wing bands. From one day to four weeks of age they were kept in heated chick battery brooders which had five decks with two compartments on each deck. The dimensions of each compartment were 10" x 36" x 36". Not more than 50 birds before two weeks of age and not more than 25 birds after two weeks of age were kept in each compartment. Two extra feeders (½" x 5" x 10") and two extra waterers were supplied to each compartment for chicks during their first week. These were placed close to the heat source, so that chicks could reach feed and water without having to move far from the heat source. They were fed commercial chick starter ration and were

kept under a 24-hour artificial light regime during the first four-week period.

Birds from four to 12 or 16 weeks of age were kept in a larger unheated rearing battery which had four decks with two compartments on each deck. The dimensions of each compartment were 13" x 36" x 40". Ten to 14 birds were kept in one compartment. Birds were fed commercial chicken grower ration and were kept under a 14-hour artificial light regime (6 a.m. to 8 p.m.).

Birds between 12 and 16 weeks of age were moved from the rearing battery to floor pens which measured 7' x 14'. Usually 20 to 25 birds were kept together in one pen. They were fed commercial chicken grower ration and were kept under a 14-hour artificial light regime (6 a.m. to 8 p.m.). Birds were also exposed to natural daylight through the windows; thus in the summer months the lighting period was slightly longer than 14 hours.

Birds were moved to individual cages at about 22 to 26 weeks of age. The dimensions were 12" x 16" x 20" for the male cages and 10" x 16" x 16" for the female cages. They were kept individually in the cages under a 14-hour artificial light regime (6 a.m. to 8 p.m.) and were fed commercial layer ration.

Artificial insemination was applied twice a week to adult birds, kept in the individual cages, for producing

experimental birds and breeding stock (Burrows and Quinn, 1935, 1937; Moultrie, 1956).

3.4 Experimental apparatus.

3.4.1 Light stimulus.

A photostimulator (Model PS 3; Grass Medical Instruments, Quincy, Massachusetts, U.S.A.; light intensity: 750,000 candle power), with frequency ranging from 1½ to 45 flashes per second (fps) was used as a source of intermittent light stimulus.

A box (16" x 16" x 16") with mirrors on the floor and on three sides, was used in some of the experiments; a clear acrylic board was used for the front wall to permit observation; the top was left open to admit the light stimulus. The light source was adjusted to approximately one foot above the back of the bird to be tested.

3.4.2 Auditory stimuli.

Two sources of auditory stimuli were used in these studies. One was a pure-tone stimulus which was produced by an electric audiogenerator (Heathkit Model 1G-72; The Heath Company, Benton, Harbor, Michigan, U.S.A.). The sound was set at a fixed intensity (about 20 decibels) and came through a loud speaker (diameter: 6") connected to an amplifier. The frequency used ranged

from 50 to 1000 cycles per second. The intensity of sound was increased gradually within a few seconds to reduce the startle effect to the birds being tested.

Another source of auditory stimulus was a complex sound which was produced by banging on a piece of wire screen (16" x 24") placed over a chick shipping box or chick battery in which the birds were kept.

3.4.3 Olfactory stimuli.

Two sources of olfactory stimuli were used in these studies. One was obtained from two sticks of Indian incense which were burned for five minutes in a box (12" x 9" x 8") with an open top and glass walls on four sides. Birds to be tested were then put into the box and the burning of incense was continued.

Another source of olfactory stimulus was a dimethylsulfide solution (CH_3SCH_3). Five cotton balls (one cubic inch) soaked with the dimethylsulfide solution were placed in the center and in the four corners of the box mentioned above. These were placed a few seconds before the birds were tested, and were left in the box during the test.

3.4.4 Heat stimulus.

Heat stimulation was obtained by using an infra-red brooder lamp (250 watts) which was hung

approximately one foot above the floor of the box described in section 3.3.3. The birds were tested only when the temperature inside the box was 75°C at the center and not lower than 65°C at the four corners.

3.4.5 Cold stimulus.

Cold stimulation was obtained by placing the box described in section 3.3.3 which contained the birds to be tested in a refrigerated room (-8°C).

3.4.6 Time recording device.

Two stopwatches were used to record the time of stimulation, seizure latency and duration, recovery period, and time intervals between tests. They were used alternately for different time recordings which came consecutively during a test.

The equipment used in electroencephalographic (EEG) studies will be described in section 3.6.

3.5 Experiments.

3.5.1 Part I: Search for a satisfactory stimulation method.

The experiments in this part were designed to study the effects of various types of external stimulation in an attempt to obtain a reliable and consistent method for inducing seizures in genetically susceptible chickens.

A total of 409 epileptic (epi epi) and 50 non-epileptic chickens were used. The group of non-epileptic chickens consisted of six epilepsy carriers (Epi epi), six normals (Epi Epi), and 38 normal birds with unknown genotype (Epi-). They were obtained from 15 hatches (Table 1) and were subjected to heat, cold, olfactory, auditory, and photic stimulation between one and 30 days of age.

The non-epileptic chickens used in these experiments were subjected to each type of stimulation under the same conditions as the epileptic chickens. They served as controls to provide evidence that no seizures could be induced in non-epileptic chickens by the type of stimulation which induced seizures in epileptic chickens.

3.5.1.1 Studies on the response of epileptic chickens to heat and cold stimulation.

The purpose of this experiment was to study the response of epileptic chickens to heat and cold stimulation.

Heat stimulation: A total of 76 epileptic and 25 normal (Epi-) chickens, obtained from four hatches (Table 1, hatch no. 3, 4, 27 and 30), were used in the heat experiment. They were tested at various ages as follows:

Hatch no.	Age (days)					
	1	3	5	7	14	28
3		5 E	5 E	14 E	7 E	
4	21 E			5 N		
	20 N					
27						14 E
30	10 E					

E = epileptic
N = normal

They were subjected to heat stimulation (see section 3.3.4) for a maximum of three minutes. Each bird was tested only once during the heat experiment. Birds which had a seizure during heat stimulation were removed to a cool place at the onset of the seizure. Those which did not respond to heat stimulation after three minutes were also removed to a cool place in order to prevent death. Chicks usually went into a coma and died after four minutes or more of heat stress. The numbers of birds which responded to heat stimulation were recorded, and the percentage of the birds responding at each age was calculated. The results were subjected to the chi-square test of homogeneity (Snedecor, 1956). The data and the results of statistical analysis are shown in Table 2.

Cold stimulation: Twenty-five epileptic and five normal (Epi-) day-old chicks (Table 1, hatch no. 6

and 30), and 27 epileptic and two normal (Epi-) chickens at 30 days of age (Table 1, hatch no. 26) were tested. They were subjected once to cold stimulation (see section 3.3.5) with no more than five birds being tested at one time. Birds were placed inside a box in a refrigerated room (-8°C) for ten minutes. They were separated from each other by hand during the test to prevent huddling in the box. The data on number of birds responding and the percentage of the birds responding at each age are shown in Table 2. Since no bird responded to cold stimulation no statistical analysis was possible.

3.5.1.2 Studies on the response of epileptic chickens to olfactory stimulation.

The olfactory experiment was designed to study the response of epileptic chickens to two different olfactory stimuli. Sixteen epileptic and two normal (Epi-) chickens (Table 1, hatch no. 1) were used. These were subjected to olfactory stimulation from one day to seven days of age at two-day intervals. Groups of four or five birds were tested twice in each testing day, once with Indian incense and once with dimethylsulfide (see section 3.3.3). A two-hour interval elapsed between tests. The experiment was conducted using the box described in section 3.3.3. The maximum time of olfactory stimulation was 20 minutes. The data on the number of

birds which responded to olfactory stimulation and the percentage of birds responding at each age are shown in Table 3. No bird responded to olfactory stimulation.

3.5.1.3 Studies on the response of epileptic chickens to auditory stimulation.

The auditory experiment consisted of two portions. The first portion was designed to study the response of epileptic chickens to two different types of auditory stimulation; one was pure-tone stimulation and the other a complex sound stimulation (see section 3.3.2). A total of 38 epileptic and four normal (Epi-) chickens, obtained from two hatches (Table 1, hatch no. 2 and 7), were used. They were tested from one day to seven days of age at two-day intervals, and from seven to 28 days of age at weekly intervals. Birds were kept in a chick battery during this 28-day testing period. They were subjected to both types of auditory stimulation on each testing day with a two-hour interval between tests. In the pure-tone test the loud-speaker was placed inside the chick battery. The frequency of the stimulus used was increased from 50 to 1000 cps (cycles per second) during the test; that is, the stimulation was started at 50 cps, and changed consecutively to 100, 200, 500 and 1000 cps after each minute during the five-minute test. In the complex sound test, the sound was produced by banging a

wire screen on top of the chick battery for five minutes. All the birds which were kept in the battery were tested together; those which responded to auditory stimulation and underwent seizures were picked up and placed in a chick box for counting purposes, and were then put back in the chick battery after the test. The data on the number of birds which responded to each type of auditory stimulation and the percentage of birds responding at each age are shown in Table 4. No birds responded to the pure-tone stimulus. The data obtained from the birds which were subjected to the complex sound stimulus at various ages were analysed by means of the chi-square test of homogeneity and the results are shown in Table 4.

The second portion of the auditory experiment was designed to compare the responses of day-old epileptic chicks to complex sound stimulation which was produced by two different methods: by banging a wire screen beside the chick battery in which the chicks were kept, in such a way that no vibration of the battery floor was created and no hand movement could be seen by the chicks, and by banging a wire screen on top of the chick battery. The responses of epileptic chicks to complex sound stimulation alone and to the combined factors of complex sound stimulation, hand movement and vibration of the battery floor, which had a startling effect on the chicks, were compared.

A total of 197 day-old epileptic chicks from matings of epileptic x epileptic, obtained from five hatches (Table 1, hatch no. 31, 33, 34, 35 and 36), were used. They were subjected once to complex sound stimulation produced by both methods, respectively, with a two-hour interval between the tests. The stimulation time was five minutes. Birds that had seizures during the test were removed from the chick battery for counting purposes.

The data on the number of birds which responded to each method of complex sound stimulation and the percentage of response from each hatch are shown in Table 5. The data on the number of birds of each hatch and the combined data from five hatches were analysed by means of the chi-square test of independence. The results of statistical analyses are shown in Table 5.

3.5.1.4 Studies on the response of epileptic chickens to photic stimulation.

The light experiment consisted of two parts. The first part was designed to study the response of day-old epileptic chicks to light conditions in the environment they were first exposed to after being removed from the hatching compartment of the incubator. A group of 114 epileptic chicks from matings of epileptic x epileptic obtained from three hatches (Table 1, hatch no.

31, 33 and 34), were removed from the darkened or dimly lighted hatching compartment of the incubator to a dark room, and were allowed to remain in the dark for five minutes of observation; those having a seizure were removed from the hatching tray to a chick shipping box for counting purposes. The remaining chicks were left in the hatching tray as before and the lights (18 fluorescent light bulbs, F 40CW) of the room were turned on; the chicks were allowed to remain in the lighted room for 20 minutes of observation; chicks undergoing seizures were removed for recording. Another group of 83 epileptic chicks (from matings of epileptic x epileptic) were taken from the same hatching compartment of the incubator into the same room with the light being turned on before introduction of the chicks; they remained in the lighted room for 20 minutes of observation, and seizure incidence was recorded. These two groups of birds were then kept in chick shipping boxes and were subjected to ILS of 14 fps two hours later; chicks were tested in groups of about five in the chick box for a maximum of three minutes. The numbers of birds which had seizures in the dark and lighted environments and during ILS were recorded and the percentage of birds having seizures was calculated. The results were analysed by means of the chi-square test of independence. The data are shown in Table 6 and the results of statistical analyses are shown in Table 7.

The second portion was designed to study the response of chickens to intermittent light stimulation (ILS). A group of 30 epileptic, six epilepsy carrier and six normal (Epi Epi) chickens (Table 1, hatch no. 20) were tested with ILS of 14 fps in a mirror box (see section 3.3.1). They were tested from one day to seven days of age at two-day intervals, and from 14 to 28 days of age at weekly intervals. Each bird was tested once on each testing day. The maximum time of ILS was three minutes. ILS was discontinued ten seconds after the beginning of a seizure. The data on the number of birds which responded to ILS and the percentage of birds responding at each age are shown in Table 8. The data were analysed by means of the chi-square test of homogeneity and the results are shown in Table 8. The results of this portion were also used as part of the results in the experiment described in section 3.5.2.1.

A comparison of the results of epileptic chickens subjected to heat, auditory and intermittent light stimulation at each age is summarized in Table 9; and the results of the chi-square test of homogeneity are shown in Table 9.

3.5.2 Part II: Studies on epileptiform seizures in chickens induced by intermittent light stimulation.

Based on its high effectiveness in inducing

seizures in epileptic chickens (Tables 6 and 8), intermittent light stimulation (ILS) was chosen as the stimulation method for further studies of epileptic chickens.

The experiments in this part were designed to study the effects of some internal and external environmental factors on the response of epileptic chickens to ILS.

3.5.2.1 The effects of age and flash frequency of intermittent light stimulation on epileptiform seizures in chickens.

In this experiment, the effects of age and flash frequency on the response of epileptic chickens to ILS were studied.

A total of 491 epileptic, 22 epilepsy carrier, and 28 normal (Epi Epi) chickens which were obtained from 23 hatches (Table 1) were used. Most of them were tested individually in a mirror box with ILS (see section 3.3.1) at frequencies of 2, 5, 8, 10, 12, 14, and 20 flashes per second (fps). They were tested usually from one to seven days of age at two-day intervals, and from two to eight weeks of age at weekly intervals, and then were tested at 26, 52 and 104 weeks of age; others were tested at frequencies of 30 and 40 fps at one day, four weeks, eight weeks, 26 weeks, 52 weeks and 104 weeks of age. The numbers of birds tested at each ILS frequency

are shown as follows:

Genotype	Numbers of birds tested with ILS of								
	2 fps	5 fps	8 fps	10 fps	12 fps	14 fps	20 fps	30 fps	40 fps
Epileptic (epi epi)	90	122	50	25	55	65	65	75	66
Carrier (Epi epi)	2	10	2	2	2	6	4	2	2
Normal (Epi Epi)	3	12	3	2	2	6	6	3	3

The maximum time of stimulation was three minutes. The stimulation was discontinued ten seconds after the beginning of a seizure. Some of the birds which were subjected to ILS frequencies of 14 and 20 fps were also subjected to ILS of 5 fps on each testing day with a two-hour interval between the tests. The epileptic chickens which were tested at one and two years of age were subjected to ILS repeatedly within a five-day period. They were tested once in the morning and once in the afternoon with one of the ILS frequencies described above at each test. Because of some technical problems and the failure of the photostimulator to function properly at certain periods of time, data were not obtainable from some of the birds tested at that time.

The normal and epilepsy carrier chickens used in this experiment were tested under the same conditions

as the epileptic chickens. They were tested from one day to seven days of age at two-day intervals, and from two to eight weeks of age at weekly intervals. They served as the controls to provide evidence that no seizure could be induced with ILS in non-epileptic chickens.

The numbers of epileptic chickens which responded to each ILS frequency at each age were recorded and the percentage of seizure susceptibility (percentage of complete seizures + percentage of incomplete seizures) was calculated. The seizure severity was classified as complete or incomplete (refer to Results section 4.1). The numbers of birds which had complete and incomplete seizures induced with each ILS frequency at each age were recorded and the percentages of complete and incomplete seizures were calculated. The data for seizure susceptibility and percentages of complete and incomplete seizures are shown in Table 10 and in Figures 4 and 5. The results of chi-square test of homogeneity was used to analyse the data of seizure susceptibility and seizure severity; the results of these analyses are shown in Tables 11 and 12.

The seizure latencies (the time recorded from the beginning of ILS to the first convulsive movement of the bird) and seizure durations (the time recorded from the first convulsive movement of the bird to the end of clonic convulsions) of epileptic chickens subjected to each

ILS frequency at each age were recorded. The distribution of seizure latencies and seizure durations of birds tested at each age with ILS of 5 to 20 fps are summarized in Tables 13 and 18. The distribution of seizure latencies and seizure durations of birds tested between one day and 104 weeks of age at each ILS frequency are summarized in Tables 14 and 19. The average seizure latency and average seizure duration of birds tested at each ILS frequency at each age are shown in Tables 15 and 20. The data of average seizure latency and duration were analysed by means of analysis of variance (Snedecor, 1956). The results of these analyses are shown in Tables 16 and 17.

3.5.2.2 The effect of sex on epileptiform seizures in chickens.

A group of 93 epileptic chickens consisting of 43 males and 50 females were obtained from six hatches (Table 1) for use in a study of sex differences in response to ILS. They were tested from one day to seven days of age at two-day intervals, from 14 to 56 days of age at weekly intervals, and were tested again at 16, 26, and 52 weeks of age. Birds were tested individually in the mirror box with ILS (see section 3.3.1). Each bird was tested twice on each testing day. Seventeen out of the 43 males and 38 out of the 50 females were tested at ILS frequencies of 5 and 14 fps, and the remaining 26 males and 22 females were tested at 5 and 20 fps, with a

two-hour interval between the tests.

The data on seizure susceptibility and seizure severity (complete and incomplete seizures) of male and female chickens are summarized in Table 21 and are shown in Figure 4. These data were subjected to the chi-square test of independence and the results of analyses are shown in Table 21.

The data on seizure latency and seizure duration are summarized in Table 22. The results of analyses of variance of these data are shown in Table 23. The distribution of seizure durations of male and female epileptic chickens are shown in Table 24; the results of the chi-square test of independence are shown in Table 24.

The results of this experiment were also used as part of the results of the experiment described in section 3.5.2.1.

3.5.2.3 The effect of parentage on epileptiform seizures in chickens.

One hundred and fifty-nine epileptic chickens obtained from nine hatches (Table 1) were used in a study of parental effect on epileptiform seizures in chickens. They consisted of 73 birds from matings of epileptic male x epileptic female, 18 from matings of epileptic male x carrier female, 37 from matings of carrier male x

epileptic female, and 31 from matings of carrier male x carrier female. They were identified by observing at least one spontaneous seizure, or a seizure induced by banging a wire screen on top of a chick shipping box in which the birds were kept at hatching day. They were tested from one day to seven days of age at two-day intervals, and from 14 to 56 days of age at weekly intervals, in a mirror box with ILS (see section 3.3.1) as follows:

Parentage			No. of birds subjected to ILS of					
Male		Female	5 fps	14 fps	20 fps	5 & 14 fps	5 & 20 fps	Total
E	x	E	15	13	14	16	15	73
E	x	C	1	0	1	8	8	18
C	x	E	6	3	0	14	14	37
C	x	C	2	3	2	13	11	31
			24	19	17	51	48	159

Birds which were subjected to ILS frequency of 5, 14 or 20 fps were tested only once on each testing day; birds which were subjected to frequencies of 5 and 14 fps, or 5 and 20 fps of ILS were tested once at each frequency on each testing day, with a two-hour interval between the tests. The maximum time of ILS was three minutes. The stimulation was discontinued ten seconds after the beginning of a seizure.

The data on seizure susceptibility and seizure severity (complete and incomplete seizures) of epileptic chickens obtained from each mating and the results of the chi-square test are summarized in Table 25.

The data on seizure latency and seizure duration of epileptic chickens obtained from each parental mating are shown in Table 26. The data were analysed by means of analysis of variance and Duncan's multiple range test (Steel and Torrie, 1960). The results of analyses are shown in Tables 26 and 27.

The results of this experiment were also used as part of the results of the experiment described in section 3.5.2.1.

3.5.2.4 The effect of prolonged intermittent light stimulation on epileptiform seizures in chickens.

The purpose of this experiment was to study the effect of prolonged ILS after a seizure had been induced on the duration of that seizure and the post-seizure recovery period of epileptic chickens.

Forty-one epileptic chickens which were obtained from four hatches (Table 1) were used. Seven of them (Table 1, hatch no. 20) were tested at 21 to 22 days, 13 (Table 1, hatch no. 18) were tested at 42 to 46 days, 13 (Table 1, hatch no. 17) were tested at 56 to 58 days, and eight (Table 1, hatch no. 15) were tested at 69 to 70

days of age. Birds were tested individually in a mirror box with ILS of 14 fps (see section 3.3.1). They were subjected to two different stimulation times: first to ILS which was discontinued ten seconds after the onset of seizure, and then during the next day to ILS which was discontinued 180 seconds after the onset of seizure.

The data on seizure latency and seizure duration, and the results of their analysis of variance are described in the Results section.

The recovery time was the time required for the bird to return to an upright position after a seizure. The data on recovery time and the results of their chi-square analyses are shown in Table 28.

3.5.2.5 The effects of some stressful conditions on epileptiform seizures in chickens.

The purpose of this experiment was to study the effects of various stressful treatments on epileptic chickens subjected to ILS of 14 fps immediately after each treatment.

One hundred and seventy-seven epileptic chickens obtained from five hatches (Table 1) were tested between one day and 54 days of age. They were subjected to the following treatments: (1) Ten day-old chicks (Table 1, hatch no. 30) and 14 chicks (Table 1, hatch no. 27) at 28 days of age were subjected to heat stress of

65° to 75°C (see section 3.3.4) for three minutes; (2) 38 day-old chicks (Table 1, hatch no. 30 and 32) and 20 birds (Table 1, hatch no. 26) at 34 and 54 days of age were subjected to cold stress of -8°C (see section 3.3.5) for ten minutes; (3) 20 birds (Table 1, hatch no. 26) at 35 days of age were subjected to ILS of 2 fps (see section 3.3.1) for ten minutes; (4) 39 day-old chicks (Table 1, hatch no. 30), 36 birds (Table 1, hatch no. 29) at 30 days of age, and 20 birds (Table 1, hatch no. 26; the same group of birds which was used in treatment 3) at 42 days of age were subjected to emotional disturbance by swinging the birds to be tested to and fro gently for one minute in a wire-mesh cage (8" x 8" x 8"). Birds which remained normal after being subjected to one of these treatments were then tested immediately in a mirror box with ILS of 14 fps (see section 3.3.1). The same group of birds which remained normal during each treatment was tested again individually two hours later with ILS of 14 fps alone to serve as the control. Birds which were subjected to the conditions in treatment 1, 3 and 4 were tested individually. Birds which were subjected to cold treatment were tested in groups of four or five birds. The maximum time of ILS (14 fps) was three minutes. ILS was discontinued ten seconds after the beginning of a seizure.

Data on birds responding to various treatments and to ILS (14 fps) at each age, and the results of the

chi-square test of independence of the data are shown in Table 29.

3.5.2.6 The response of epileptic chickens to successive intermittent light stimulations at various time intervals.

This experiment was designed to study the time intervals following a seizure required for epileptic chickens to regain their ability to produce a seizure. The response of epileptic chickens which had previously had a seizure induced by ILS to successive ILS at various time intervals was studied.

This experiment was divided into two parts. The first part consisted of subjecting epileptic chickens to two successive intermittent light stimulations which were separated by various time intervals. The ILS frequencies used were 5, 14 and 20 fps, and the time intervals used were 0, 5, 15, 30 and 60 minutes. Three groups of epileptic chickens were used. They were identified by showing at least one spontaneous seizure or a seizure induced by ILS. The first group (Table 1, hatch no. 28) and the second group (Table 1, hatch no. 26) each consisted of 25 epileptic chickens, and the third group (Table 1, hatch no. 27) consisted of 30 epileptic chickens. Chicks were tested individually in a mirror box (see section 3.3.1) at one day, four weeks and eight weeks of age.

At one day of age, each group of birds was

subjected to two successive intermittent light stimulations of the same frequency. The first group of birds was subjected to 5 fps, the second group to 14 fps, and the third group to 20 fps. Each bird was subjected to one of the five time interval tests.

At four and eight weeks of age, each group of birds was subjected to two frequencies of intermittent light stimulation to increase the number of birds tested at each frequency of ILS at each time interval, and to substitute for the birds which died before four or eight weeks of age or birds which did not respond to ILS on the testing days; the first group of birds was subjected to 5 and 20 fps, the second group was subjected to 5 and 14 fps, and the third group was subjected to 14 and 20 fps of ILS on two successive days, with one frequency of ILS on one day, and the other frequency of ILS on the following day. One of the five time intervals was used on each bird during each testing day.

The numbers of birds which were tested at each ILS frequency at each time interval and at each age are shown as follows:

ILS (fps)		Time interval following seizure (mins.)				
		0	5	15	30	60
5	1 day	5	5	5	5	5
	4 weeks	10	8	9	8	7
	8 weeks	5	5	5	5	5
14	1 day	5	5	5	5	5
	4 weeks	11	10	11	10	11
	8 weeks	5	5	5	5	5
20	1 day	6	6	6	6	6
	4 weeks	10	10	10	10	9
	8 weeks	5	5	5	5	5

The data for birds responding to the second stimulation at each ILS frequency at each time interval are shown in Table 30. The number of birds tested at each time interval was too small for statistical analysis, thus the data for birds subjected to three ILS frequencies at each time interval were combined and were analysed by means of the chi-square test of homogeneity; the combined data and the results of analyses are shown in Table 30.

The data for average latencies and durations of the first and second seizures in birds subjected to each ILS frequency at each time interval are shown in Table 31; the data were analysed by means of the analysis of variance and the results of analyses are shown in Table 32.

The second part of this experiment was designed to study the length of rest between seizures required for epileptic chickens to undergo repeated seizures. Birds used in this part were the same as those used in the first part of the experiment. They were tested at four and eight weeks of age with ILS of 14 fps (see section 3.3.1). They were subjected to a series of stimulations with a maximum time of three minutes for each ILS; stimulation was discontinued ten seconds after the beginning of a seizure. At four weeks of age, ten birds were tested at 15-minute intervals between stimulations and five birds were tested at 30-minute intervals between stimulations. At eight weeks of age, ten birds were tested at five-minute intervals and five birds were tested at 15-minute intervals between stimulations. Birds were tested individually and the test ended when the bird had been subjected to a series of eight stimulations or ended when the bird being tested failed to respond to the stimulus before eight successive stimulations had been completed. The number of seizures each bird experienced and the latency and duration of each seizure were recorded. The data are shown in Table 33 and Table 34. Because of inadequate Sample Sizes no statistical analysis was performed.

3.5.3 Part III: Genetic studies on segregation of the *epi* gene and its effect on sex distribution, fertility, embryonic mortality and hatchability.

Segregation of the *epi* gene and sex distribution in chicks, and fertility, embryonic mortality and hatchability of eggs obtained from various matings were observed. The birds which were used for producing the eggs and chicks for this experiment were all selected from the P_2 and P_3 breeding stocks. They were crossed by means of artificial insemination twice a week using pooled semen. The matings were as follows: five epileptic males x five epileptic and eight carrier females, five carrier males x eight epileptic and 19 carrier females, and five normal males x five epileptic and six normal females. Approximately 0.05 ml. of semen was inseminated to each female within 30 minutes following semen collection. Eggs from each female were marked with the cage number and date of production. They were collected every day and were kept in a cool room at a temperature of 8°C. They were set in an incubator every week and were candled at the fifth and seventeenth days of incubation, and were transferred into the hatching compartment of the incubator at the seventeenth day of incubation. A total of 1312 eggs were set in 12 hatches. Fertility, embryonic mortality and hatchability were recorded. Infertile eggs were broken open and examined macroscopically to verify infertility.

Nine hundred and seventy-five chicks were

obtained from these 12 hatches (Table 1). They were placed in chick shipping boxes as soon as they were removed from the incubator. Chicks with different parentages were placed separately in different boxes. They were subjected to three tests by means of ILS of 14 fps (see section 3.3.1) with a two-hour interval between the tests. The maximum time of ILS was three minutes. The numbers of birds which responded to ILS were recorded. After three tests all the chicks were sacrificed for sexing. The sex of each chick was determined by the presence of testes or the left oviduct in the abdominal cavity.

The data on epi gene segregation are shown in Table 35 and the data on sex distribution are shown in Table 36; these data were analysed by means of heterogeneity chi-square (Snedecor, 1956) and results of the analyses are shown in the same tables.

The data on fertility, embryonic mortality and hatchability are shown in Table 37; the data were analysed by means of the chi-square test of homogeneity and the results are shown in the same table.

3.6 Part IV: Electroencephalographic (EEG) studies.

Twelve epileptic (three male and nine female), five carrier (two male and three female), and four normal (one male and three female) chickens obtained from

four hatches (Table 1) were used for EEG studies. More female birds were used in the studies because they had a smaller comb which was convenient for implanation and for EEG recording.

The implanting technique was recommended by Dr. E.C. Crichlow, Associate Professor, Department of Veterinary Physiology, Western College of Veterinary Medicine, University of Saskatchewan. Steel electrodes were implanted in the birds between three and four months of age. Dr. Crichlow supervised all implanting operations. The feathers on the head region were removed by hair clippers. The head region of the bird to be implanted was immobilized by a chicken head holder (Chicken Adaptor Model 1217, David Kopf Instruments, Tujunga, California, U.S.A.). The top and side regions of the bird's head were anesthetized locally with Nembutal. The skin was dissected to expose the skull and each electrode was inserted into a small hole drilled into the skull. The holes were made without piercing the dura. The locations of the electrodes are shown in Figure 1. They were implanted bilaterally near the edge of the frontal bone, two at the anterior end, two at the middle, and two at the posterior end. The electrodes were then fastened with dental cement. The skin was sutured back around the electrodes. After the operation, the birds were housed individually or in pairs in cages measuring 15" x 18" x 18".

EEG recordings could be made beginning 24 hours after the implantation.

The brain waves of each bird were recorded by an eight-channel EEG recording machine (Model III D, Grass Instrument Company, Quincy, Massachusetts, U.S.A.).

The EEG recording apparatus is illustrated in Figure 2. The bird was held inside a duplex box made of two boxes with one placed on top of the other. The floor of the upper observation box (13" x 22" x 10") had been removed and the box was placed on top of the supporting box (14" x 22" x 10"). A piece of one-way mirror was placed in the front wall of the observation box. The light source was placed on top of the observation box over a round opening (diameter = 5"). The electrodes on the chicken's skull were connected by an adaptor to a cable leading to the EEG recording machine, the cable passing through a small hole on top of the observation box. The bird to be studied was suspended between upper and lower chambers by a cloth harness. Wings and body were restrained by the harness but legs and head were free.

The recording was done in a dimly lit room to reduce unnecessary distraction to the bird. The resting EEG of the bird was recorded first. The bird was then subjected to ILS for 60 seconds or more. ILS frequencies of 5, 8, 10, 12, 14 and 20 fps were used. The bird was

stimulated with only one ILS frequency during the recording session.

Tracings of typical resting EEG and EEG during ILS for epileptic, carrier, and normal chickens are shown in Figure 7.

The abnormal EEG tracings which were associated with the initial response of epileptic birds to different ILS frequencies during the recording process are shown in Figure 8.

Data on frequency and amplitude of resting EEG and of EEG during ILS of epileptics (combined results of 12 birds), carriers (combined results of five birds), and normals (combined results of four birds) are summarized in Table 38. Frequency and amplitude of the brain waves in each chicken at each lead were the average measurement of a period of five seconds of EEG recording. The data were subjected to a one-tailed t-test (Steel and Torrie, 1960) and the results of the t-test are shown in Table 39.

4. RESULTS

4.1 General description of photogenic and audiogenic seizures in chickens.

In this section, a general description of the seizure patterns is presented. The patterns described are those observed during the course of experimental work from 1969 to 1971. Two different degrees of seizure severity have been observed repeatedly; these are classified as complete or incomplete seizures. It has also been noted that seizure pattern in response to auditory stimulation differs from that induced by ILS.

The typical pattern of light-induced complete seizures in chickens is shown in Figure 3. Pictures in the figure show the sequence of a complete seizure in an 11-day-old epileptic chick induced by ILS at a frequency of 14 flashes per second (fps). At the beginning of the seizure, the individual stands quietly for several seconds. Then the head begins to turn upwards, backwards, or to either side of the body. The individual may turn around several times. The wings, stretched outwards or upwards, begin to flap. The individual falls to the floor with wings and legs thrashing violently. Pecking movements of the beak usually occur during the seizure. The individual may get up and run or dash for some distance, falling down again and continuing with the convulsive movements; this

may be repeated several times during the seizure. Clonic muscular contractions are prominent during the seizure. However, one or a few very brief tonic-like convulsive movements may be seen between the clonic convulsive movements; the tonic-like convulsive movements are characterized by the wings stretching upwards and outwards, and the legs stretching backwards and trembling. The attack usually ceases abruptly. The individual may return to a normal standing position immediately after the seizure, or may remain lying on the floor for several seconds to more than half an hour, with the eyes open or closed and the legs trembling slightly. The bird eventually returns to normal. A coma occasionally occurs following a seizure.

The pattern of audiogenic seizures is quite similar to that induced by ILS, except that at the beginning of a seizure the individual which is subjected to auditory stimulation has a very short moment of alarm or startle response, followed by screaming and running wildly across the floor apparently searching for a hiding place; the individual eventually falls to the floor and enters a convulsion as described above in light-induced seizures.

Coma is not common in chickens with epileptiform seizures. It occurs mostly in day-old chicks and seldom appears in older birds. More than 3000 ILS-induced seizures have been observed in birds, varying in age from day-old to two years. Thirty-two out of the 670 day-old

epileptic chicks were found to have a seizure followed by a coma, but only two out of the 219 birds tested at 21 days of age, one out of 284 birds tested at 28 days of age, and four out of the 253 birds tested at 56 days of age had a seizure followed with a coma.

Incomplete seizures have been observed in affected chickens during ILS but not during auditory stimulation. The head turns upwards or to one side of the body, and the individual may turn around several times; wings may stretch upwards or outwards and may flap several times; then the bird returns to normal and stands quietly. The individual may remain unaffected by continued ILS, the same response may reappear once or repeatedly during the stimulating period, or a complete seizure may occur in the latter part of the stimulating period.

4.2 Part I: Response of epileptic chickens to different types of stimulation.

4.2.1 Response of epileptic chickens to heat and cold stimulation.

The data on the response of birds to heat and cold stimulation at different ages are shown in Table 2.

The epileptic and normal chickens which were subjected to heat stimulation responded to the stimulus after a few seconds by running, screaming and panting inside the box, but only the epileptic birds fell to the floor and underwent seizures within two minutes of the

start of stimulation. Both epileptic and normal chickens went into a coma and died if they were subjected to heat stimulation for more than four minutes. The susceptibility of epileptic chickens to heat stimulation was significantly affected by age ($P<0.01$); 60 to 100 per cent of the epileptic birds tested during their first week of life had seizures during heat stimulation, but the incidence of seizures decreased to 28.6 per cent in birds tested at 14 and 28 days of age (Table 2).

Twenty normal chicks at one day of age and five at seven days of age were subjected to heat stimulation; none of them had seizures during heat stimulation (Table 2).

No epileptic or normal chickens which were tested at one day and 30 days of age had seizures during cold stimulation (Table 2); their only response was peeping and huddling together in the box.

4.2.2 Response of epileptic chickens to olfactory stimulation.

No epileptic or normal chickens which were tested from one to seven days of age at two-day intervals were found to respond to olfactory stimulation; they remained normal when subjected to the smell of Indian incense and to the smell of dimethylsulfide (Table 3).

4.2.3 Response of epileptic chickens to auditory stimulation.

Epileptic seizures could not be induced from the epileptic or normal chickens by using a pure-tone stimulus; but epileptic chickens responded to complex sound stimulation which was produced by banging a wire screen on top of the chick battery in which they were kept (Table 4). The susceptibility (% of response) of epileptic chickens to the complex sound stimulus was significantly affected by age ($P<0.01$). About 60.5 per cent of the day-old epileptic chicks had seizures induced by complex sound stimulation. The incidence of seizures declined to 47.4, 21.0, and 6.1 per cent in birds tested at three, five and seven days of age, respectively. However, the susceptibility of the epileptic chickens increased with age; the incidence of seizures was 39.4, 61.3, and 64.5 per cent in birds tested at 14, 21, and 28 days of age, respectively. None of the normal chickens was found to have seizures induced by complex sound stimulation.

The data for comparative study of the responses of day-old epileptic chickens to two different methods of complex sound stimulation are shown in Table 5. Birds were subjected to complex sound produced by banging a wire screen beside the chick battery and to the combined effect of complex sound, movement and vibration which was produced

by banging a wire screen on top of the chick battery in such a way that the operator and his hand movements could be seen by the birds (see section 3.5.1.3). The results showed that complex sound stimulation alone was not the major factor causing epileptic chickens to undergo seizures; the seizures were more likely due to the combined effect of sound, movement and vibration which created emotional or startle response in epileptic chickens. In 197 day-old epileptic chickens (combined data of five hatches) which were subjected to both methods of complex sound stimulation respectively, 45.2 per cent of the chicks responded to complex sound stimulation which was produced by banging a wire screen on top of the chick battery, but only 5.6 per cent of them had seizures when complex sound stimulation was produced by banging the wire screen beside the chick battery.

The results of statistical analyses showed that the differences in percentages of birds in each hatch and in the combined data of five hatches which had seizures induced by different methods of complex sound stimulation were highly significant ($P<0.01$) except the results obtained from birds in hatch no. 35 (Table 5). In hatch no. 35, although the difference between the percentages of responses was not statistically significant, the percentage of birds which responded to complex sound stimulation produced by banging the wire screen on top of the chick battery (37.1

per cent) was much higher than the percentage of birds which responded to complex sound stimulation produced by banging the wire screen beside the chick battery (17.1 per cent).

4.2.4 Response of epileptic chickens to photic stimulation.

The data on the response of newly hatched epileptic chicks to their first environmental light exposure are shown in Table 6.

The results show that only 0.9 per cent of the 114 epileptic chicks (combined data of three hatches) had seizures when they were removed from the darkened or dimly lighted hatching compartment of the incubator into a dark room, but 79.5 per cent of the 83 epileptic chicks (combined data of two hatches) had seizures when they were removed from the same hatching compartment of the incubator into the same room with light. Of the 113 epileptic chicks in the former group which remained normal in the dark room and did not have seizures during the five-minute period of observation, 58.4 per cent had seizures soon after turning on the light. All of these epileptic chicks were shown to be highly susceptible to seizures by their response to intermittent light stimulation (ILS); 93.4 per cent of them (combined data of five hatches) had seizures when they were subjected to ILS of 14 fps.

The results of statistical analyses are shown in

Table 7. The percentages of birds which responded to ILS were significantly higher than those which were left in a dark room, those left in a dark room but with light being turned on after five minutes, and those which were moved into a brightly lit room, except in hatch no. 35. The percentage of birds which responded to ILS (77.1 per cent) was only slightly higher than the percentage of birds which responded to the bright light (74.3 per cent) in hatch no. 35, and the difference was not statistically significant (Table 7). The percentages of birds which had seizures in the lighted room were significantly higher than the percentages of birds which had seizures in the dark room or in the lighted room after they had remained normal in the dark for five minutes; the percentages of birds which had seizures in the lighted room after remaining normal in the dark for five minutes were significantly higher than the percentages of birds which had seizures in the dark room.

Intermittent light stimulation (ILS) was found not only highly effective for inducing seizures from newly hatched epileptic chickens, but was also highly effective for birds at other ages except for birds tested at three to seven days of age. The statistical analysis showed that the susceptibility of birds was significantly affected by age ($P<0.01$); all the epileptic chicks tested at day-old and 92 to 100 per cent of the birds tested from 14 to

28 days of age had seizures induced by ILS of 14 fps, but the incidence of seizures was markedly reduced in birds tested during three to seven days of age; only 36.7, 46.4, and 48.0 per cent of the birds which were tested at three, five and seven days of age respectively, responded to ILS (Table 8). No epilepsy carrier or normal chickens which were tested in the same way as epileptic chickens had seizures during ILS.

4.2.5 Comparison of epileptic chickens responding to heat, auditory and intermittent light stimulation.

The data on comparison of percentages of epileptic chickens which responded to heat, complex sound and intermittent light stimulation are shown in Table 9. The differences between percentages of birds which responded to these stimuli at each age were highly significant ($P<0.01$) except in birds tested at three days of age. ILS seemed to be more effective and more reliable for inducing seizures from epileptic chickens than sound stimulation, and was found relatively more effective than heat stimulation for inducing seizures from epileptic chickens tested at day-old and after seven days of age, but was less effective than heat stimulation in birds tested at five and seven days of age. Sound stimulation seemed to be more effective than heat for inducing seizures from older epileptic chickens.

4.3 Part II: Studies on epileptiform seizures in chickens induced by intermittent light stimulation.

4.3.1 The effects of age and flash frequency of intermittent light stimulation on epileptiform seizures in chickens.

4.3.1.1 Seizure susceptibility and seizure severity.

The data on response of epileptic chickens to various intermittent light stimulation (ILS) frequencies at different ages are shown in Table 10 and in Figures 4 and 5. Analyses of data are shown in Tables 11 and 12.

The seizure susceptibilities (per cent of complete seizures + per cent of incomplete seizures) of epileptic chickens subjected to various ILS frequencies were significantly affected by age ($P<0.01$) except in birds which were subjected to 30 fps of ILS (Table 11).

The ILS of 2 fps was found to be effective for inducing seizures in day-old chicks, but had little or no effect on birds tested at older ages (Table 10 and Figure 4). At one day of age 97.5 per cent of the epileptic chicks responded to 2 fps of ILS, the incidence of seizures declined to less than 20 per cent after one day of age, and chicks seldom responded to this frequency of ILS after five days of age.

Of the epileptic chickens which were subjected to ILS of 5 to 20 fps at early ages, the seizure susceptibility declined sharply during three to seven days of age (Figure 5); the incidence of seizures ranged from

20.3 to 50.9 per cent at these ages except that at seven days of age 78.5 per cent of the birds tested with 8 fps of ILS had seizures. However, the susceptibility increased rapidly after seven days of age and remained relatively high after that. The incidence of seizures remained at 77 to 100 per cent in birds tested at or after two weeks of age at ILS frequencies of 8 to 20 fps and in birds tested after six weeks of age at a frequency of 5 fps. Of epileptic birds which were subjected to 30 and 40 fps of ILS, the seizure susceptibility increased rapidly from eight weeks of age; all the birds responded to 30 and 40 fps of ILS at 52 and 104 weeks of age (Figure 4 and Table 10).

The seizure susceptibilities were significantly different in birds tested with various frequencies of ILS at each age except in birds tested at three days and 104 weeks of age (Tables 10 and 12).

In general, the majority of birds which responded to ILS had complete seizures. A total of 3199 seizures were obtained from 491 epileptic birds tested at between one day and 104 weeks of age with ILS of 2 to 40 fps, 87 per cent of which were complete seizures.

The incidence of complete seizures in birds subjected to ILS of 5 to 20 fps was high at day-old, declined sharply during three to seven days of age, and then increased rapidly after seven days of age (Figure 5);

it increased relatively slowly in birds tested at frequencies lower than 10 fps. In birds tested with 30 and 40 fps of ILS, the incidence of complete seizures was relatively lower at four weeks of age than at one day of age, and increased rapidly after four weeks of age (Table 10). All of the birds which responded to ILS at 52 and 104 weeks of age had complete seizures except that five per cent of the birds which responded to 8 fps of ILS at 52 weeks of age had incomplete seizures.

The differences in the percentages of complete seizures which were obtained from birds tested with various frequencies of ILS at each age were highly significant ($P<0.01$) except in birds tested at five, 52 and 104 weeks of age (Tables 10 and 12).

Incomplete seizures were not common in day-old chicks; not more than five per cent of the day-old epileptic chicks which responded to ILS had incomplete seizures. However, birds which were tested with 30 fps of ILS had an incidence of incomplete seizures of 12.1 per cent (Table 10). Incomplete seizures occurred more often in birds tested between three days and 26 weeks of age; the incidence of incomplete seizures in birds tested with ILS of 5 to 40 fps ranged from zero (in birds tested at 14 and 20 fps) to 33.3 per cent. Incomplete seizures were also less common in birds tested at and after 52 weeks of age; only five per cent of the birds tested at 8 fps of

ILS at 52 weeks of age had incomplete seizures and none were observed at 104 weeks of age.

The differences between the percentages of incomplete seizures which were obtained from birds subjected to various frequencies of ILS at each age were mostly non-significant except from birds tested at one day, four weeks, seven weeks, and 26 weeks of age (Table 12). However, after six weeks of age incomplete seizures occurred less often in birds tested at 14 and 20 fps than in birds tested at frequencies of lower and higher than 14 and 20 fps of ILS.

The most effective flash frequency of ILS for inducing seizures from epileptic chickens, based on the results of seizure susceptibility and the percentage of complete seizures, was 10 to 20 fps in birds tested between one day and eight weeks of age, and 10 to 40 fps in birds tested between 26 and 52 weeks of age; all the birds responded to ILS of 5 to 40 fps with complete seizures at 104 weeks of age (Table 10 and Figures 4 and 5).

The results of normal (Epi Epi) and epilepsy carrier (Epi epi) chickens which were tested under the same conditions as the epileptic chickens are shown as follows:

		Intermittent light stimulation (fps)								
		2	5	8	10	12	14	20	30	40
No. of birds tested	Normal:	3	12	3	2	2	6	6	3	3
	Carrier:	2	10	2	2	2	6	4	2	2
No. of birds responding during the eight-week testing period	Normal:	0	0	0	0	0	0	0	0	0
	Carrier:	0	0	0	0	0	0	0	0	0

They were tested from one day to seven days of age at two-day intervals, and from two weeks to eight weeks of age at weekly intervals with ILS of 2 to 20 fps, and at one day, four weeks and eight weeks of age with ILS of 30 and 40 fps. The normal and carrier chickens which were subjected to ILS of 14 and 20 fps were also subjected to ILS of 5 fps on each testing day with a two-hour interval between the tests. None of the normal or carrier chickens had seizures induced with ILS between one day and eight weeks of age.

4.3.1.2 Seizure latency.

The seizure latency of incomplete seizures was usually difficult to determine because the initial symptoms of incomplete seizures were hard to identify and responses occurred intermittently at various intervals in the same trial. The latency of incomplete seizures,

as well as could be determined, ranged from ten to 175 seconds.

The latency of complete seizures ranged from five to 120 seconds with the distribution skew towards the lower end (Tables 13 and 14). The distribution of seizure latencies of epileptic chickens tested at various ages between one day and 104 weeks of age are shown in Table 13; the distribution of seizure latencies of epileptic chickens tested at various ILS frequencies of 5 to 20 fps are shown in Table 14. (The data shown in Tables 13 and 14 were based on the combined data of birds tested between one day and 104 weeks of age with ILS of 5 to 20 fps, because the data on birds tested with ILS of 2, 30 and 40 fps were not obtainable at some ages). The average latency of birds which were subjected to ILS frequencies of 5 to 40 fps, at various ages from one day to 104 weeks of age, are shown in Table 15.

From the results of a total of 2490 seizures recorded and shown in Tables 13 and 14, about four per cent of the seizures were induced by ten seconds or less of ILS, 76.6 per cent were induced between 11 and 30 seconds of ILS, 18.7 per cent were induced by 31 to 60 seconds of ILS, and only 0.6 per cent of the seizures required more than 60 seconds of induction.

Epileptic chickens tended to have more seizures with shorter latency as the age increased; Table 13 shows

that 38.2 per cent of the seizures induced from day-old chicks had latency of 20 seconds or less, as compared to 16.9 to 22.3 per cent from birds tested at three to seven days of age; the incidence of seizures with shorter latency (20 seconds or less) increased consistently from 28.7 per cent in birds tested at two weeks of age to 52.4 per cent in birds tested at eight weeks of age, and to 100 per cent in birds tested at 104 weeks of age. The incidence of seizures with latency of ten seconds or less was low in birds tested before eight weeks of age; it ranged from zero to 3.8 per cent in birds tested between one day and eight weeks of age, but increased rapidly in birds tested beyond eight weeks of age to as high as 54.2 per cent in birds tested at 104 weeks of age. Birds tested at three to seven days of age had a higher percentage of seizures with longer latency (over 20 seconds) than those tested at other ages; 39.5 to 44.4 per cent of the seizures were induced with more than 30 seconds of ILS in birds tested at three to seven days of age as compared to less than 31 per cent in birds tested at one day and at three to eight weeks of age, and less than four per cent in birds tested after eight weeks of age. Only 0.6 per cent of the 2490 seizures were induced by longer than 60 seconds of ILS; half of them were obtained from birds tested during three to seven days of age, and the rest of the seizures were obtained from birds tested between three and eight weeks of age.

The average latencies of seizures of birds induced by various ILS frequencies at different ages are shown in Table 15; the average seizure latencies obtained from birds tested during three to seven days of age were relatively longer compared to the average seizure latencies obtained from birds tested at other ages. The average latency of seizures in birds tested after seven days of age gradually decreased with increasing age; the rate of decrease in length of average seizure latency was slightly faster in birds tested with ILS frequencies higher than 10 fps. At 104 weeks of age the average time needed for inducing a seizure with ILS had diminished to approximately one half or less of the average time needed for inducing a seizure from day-old chicks, except in birds tested at 5 fps of ILS. At 104 weeks of age the average time needed for inducing a seizure with ILS of 5 fps had diminished to approximately two-thirds of the average time needed for inducing a seizure from day-old chicks. The results of statistical analyses showed that age differences in seizure latencies of birds induced by each ILS frequency were all significant at the one per cent level (Table 16).

The seizure latency of epileptic chickens was also affected by flash frequency of ILS. The percentage of seizures with longer latency (over 20 seconds) was high in birds subjected to low frequencies of ILS; about 82.2 per cent of the seizures which were induced with ILS of

5 fps had a latency of more than 20 seconds, but the percentage gradually decreased to 69.1, 61.2, 55.0, 38.4, and 28.1 per cent of those induced with ILS of 8, 10, 12, 14 and 20 fps, respectively (Table 14).

The average seizure latencies in birds induced with various ILS frequencies at different ages are shown in Table 15. The average seizure latencies of birds induced with ILS of 5 fps were usually longer than those induced by higher ILS frequencies. The average seizure latencies of birds induced with ILS of more than 10 fps were relatively shorter than those induced with ILS frequencies lower than 12 fps at most ages. The results of statistical analyses showed that the differences between the average seizure latencies obtained from birds which were subjected to various ILS frequencies were highly significant at the one per cent level except in birds tested at three and seven days of age (Table 17).

4.3.1.3 Seizure duration.

The duration of incomplete seizures ranged from five to 95 seconds, based on data from birds which had only one response during intermittent light stimulation. Birds might have two or more responses occurring intermittently, or might eventually end up with a complete seizure in the same trial.

The duration of complete seizures ranged from

12 seconds to 5400 seconds. The distribution of seizure durations (based on the combined data of birds tested with ILS of 5 to 20 fps) of epileptic chickens tested at various ages between one day and 104 weeks of age are shown in Table 18. The distribution of seizure durations (based on the combined data of birds tested between one day and 104 weeks of age) of epileptic chickens tested at various ILS frequencies of 5 to 20 fps are shown in Table 19. The average seizure durations of birds which were subjected to various ILS frequencies of 5 to 40 fps at various ages from one day to 104 weeks of age are shown in Table 20.

Of 2490 observed seizures it was found that more than 90 per cent of them had durations under 120 seconds, and 66.9 per cent ended within 60 seconds (Tables 18 and 19); only 5.2 per cent of the seizures lasted between 121 and 600 seconds, and 1.1 per cent of the seizures lasted longer than 600 seconds.

Prolonged seizures (lasting more than 120 seconds), as well as incidence of coma (see Results section 4.1), occurred mostly in day-old chicks (Table 18); 24.6 per cent of the seizures which were induced from day-old chicks lasted between 121 and 600 seconds and about 5.9 per cent lasted longer than 600 seconds. No prolonged seizures were observed in birds tested during three to seven days of age, and less than five per cent prolonged seizures occurred in birds tested after seven

days of age. Of birds subjected to various ILS frequencies at one day of age, the average seizure durations were mostly longer than 100 seconds except for those obtained from birds subjected to ILS of 2, 12 and 40 fps (Table 20). The average seizure durations were shorter in birds tested during three to seven days of age and at or after 52 weeks of age, compared to those obtained from birds tested at one day and between two and 26 weeks of age (Table 20). The incidence of seizures with durations lasting less than 60 seconds increased as age increased (Table 18).

The results of statistical analyses showed that effects of age differences on seizure duration of birds tested at various frequencies of ILS were highly significant, except for those subjected to ILS of 12 and 40 fps (Table 16). The differences between seizure durations of birds subjected to various frequencies of ILS at each age were not statistically significant except at one day and at four, six and seven weeks of age (Table 17).

The standard errors differ greatly among some samples in the data on seizure duration. Thus the results of analyses of variance used to interpretate the data on average seizure duration in this section and in later sections may be biased due to differences in standard errors between samples.

4.3.2 The effect of sex on epileptiform seizures in chickens.

Epileptiform seizures were found in both male and female chickens. Data on male and female chickens which were subjected to ILS of 5, 14 and 20 fps at one day to 52 weeks of age are shown in Figure 6. The data and the results of statistical analyses on seizure susceptibility, and on percentages of complete and incomplete seizures are shown in Table 21. The average seizure latency and average seizure duration of male and female chickens tested between one day and 52 weeks of age at ILS frequencies of 5, 14 and 20 fps are summarized in Table 22.

Male and female chickens were similar to each other in seizure susceptibility and incidence of complete seizures, and both sexes had a relatively low percentage of incomplete seizures (Figure 6). The percentages of seizure susceptibility and complete seizures were high at one day of age, decreased sharply during three to seven days, rapidly increased by two weeks of age, and remained relatively high thereafter. In birds tested with ILS of 5 fps the seizure susceptibility and incidence of complete seizures increased slower than at other frequencies and fluctuated more with age in both sexes.

The results of statistical analyses showed that there were no significant differences in the percentages

of seizure susceptibility, complete seizures, and incomplete seizures (Table 21) and in average seizure latencies (Table 23) between male and female chickens. However, the seizure duration tended to be longer in males than in females (Tables 22 and 23). The difference in the average seizure durations between males and females was significant in birds subjected to ILS of 5 fps ($P<0.05$)(Table 23). The differences between average seizure durations of male and female chickens subjected to ILS of 14 and 20 fps were not statistically significant although they were large; the average seizure durations in males subjected to 14 and 20 fps were 65.3 $\pm$ 8.9 seconds and 93.2 $\pm$ 7.4 seconds compared to 55.5 $\pm$ 3.0 seconds and 62.9 $\pm$ 4.5 seconds in females, respectively.

Table 24 shows the distribution of seizure durations of 738 seizures obtained from male chickens and 819 seizures obtained from female chickens tested between one day and 52 weeks of age with ILS of 5, 14 and 20 fps; 69.8 to 70.9 per cent of the seizures induced in male and female chickens ended within 60 seconds and 22.4 to 24.3 per cent of seizures lasted between 61 and 120 seconds. Males tended to have a higher incidence of prolonged seizures which lasted longer than 120 seconds. Ten males and one female had seizures lasting between 991 and 5400 seconds; the difference in numbers of male and female chickens was found to be statistically significant at the

one per cent level (Table 24).

4.3.3 The effect of parentage on epileptiform seizures in chickens.

The responses of birds which were obtained from various parental crosses to ILS of 5, 14 and 20 fps between one day and eight weeks of age are summarized in Table 25. The results of statistical analyses of data on seizure susceptibility, and on incidence of complete and incomplete seizures are shown in the same table. The average seizure latency and average seizure duration are shown in Table 26 and the results of analyses are shown in Table 27.

The data obtained from progeny of different matings were not consistent in response to ILS of 5, 14 and 20 fps (Tables 25 and 26). However, in comparing the seizure susceptibility and the incidence of complete seizures the lowest percentages were often obtained from progeny produced by epileptic sires, and the highest percentages often obtained from progeny of carrier sires (Table 25). In comparing the average seizure latency the longest average seizure latency always belonged to the progeny of epileptic x epileptic birds, and the shortest average seizure latency always belonged to the progeny of carrier sires (Table 26).

In birds tested with ILS of 5 fps the seizure susceptibility and the incidence of complete seizures were

highest (71.1 and 60.4 per cent) in the progeny of carrier x carrier birds, and were lowest (48.9 and 39.8 per cent) in the progeny of epileptic males x carrier females (Table 25). The seizure susceptibility and incidence of complete seizures in the progeny of epileptic x epileptic and carrier male x epileptic birds fell between the former two groups. The analyses showed that the differences were highly significant ($P<0.01$). The incidence of incomplete seizures in the progeny from all four mutant matings fell between 9.1 and 11.3 per cent and the differences were not statistically significant. The average seizure latencies were not significantly different among the progenies of different mutant matings (Table 27), but the average latency was highest in the progeny of epileptic birds and lowest in the progeny of carrier x carrier birds (Table 26).

In birds tested with ILS of 14 fps, the differences in seizure susceptibility and incidence of complete and incomplete seizures (Table 25) and average seizure durations (Table 26) among the progenies of different matings were not statistically significant (Tables 25 and 27); but seizure susceptibility and incidence of complete seizures obtained from the progeny of carrier sires were relatively higher than those obtained from the progeny of epileptic sires. The results of analyses showed that there were significant differences in average latency among the progenies of different matings; the

results of Duncan's new multiple range test showed that the differences were mainly due to the long average latency in the epileptic x epileptic mating and to the short average latency in the carrier x carrier mating.

In birds tested with ILS of 20 fps there were significant differences in seizure susceptibility and in incidence of complete and incomplete seizures ($P<0.01$) among the progenies of different matings (Table 25). Seizure susceptibility was obviously lower in the progeny of epileptic x epileptic matings and incidence of complete seizures was obviously lower in the progeny of epileptic x epileptic and epileptic male x carrier female. No significant differences were found in the average seizure latencies and average seizure durations among the progeny of different matings (Table 27).

4.3.4 The effect of prolonged intermittent light stimulation on epileptiform seizures in chickens.

The continuation of ILS up to 180 seconds after the initiation of a complete seizure did not seem to affect the duration of that seizure. The average latency and duration of seizures obtained from the prolonged stimulation group and control group are shown as follows:

	Prolonged stimulation		Control		F ratio for analysis of variance
	Mean	SE	Mean	SE	
Average latency (sec.)	20.6	0.9	20.4	0.9	0.03
Average duration (sec.)	58.8	9.2	59.4	13.8	0.001

The differences in latencies and duration of seizures between the prolonged stimulation group and the control group were not significant at the 5 per cent level.

Depression lasting from 20 seconds to more than 30 minutes often occurred immediately after the end of a seizure in epileptic chickens; the birds lay motionless on the floor on their sides, with eyes opened or closed. The incidence of depression was found to be affected by prolonged ILS; 92.7 per cent of the birds which were subjected to prolonged ILS showed various degrees of depression compared to only 41.5 per cent in the controls; the difference was highly significant (Table 28). Birds which showed no depression regained an upright standing or crouching position immediately after the end of the seizure. The duration of depression usually lasted less than ten minutes in both prolonged stimulation and control groups; 52.6 per cent and 58.8 per cent of the seizures were followed by depression which lasted less than five minutes, 34.2 per cent and 29.4 per cent of the seizures were followed by depression which lasted between five and ten minutes, and only 13.2 per cent and 11.8 per cent of the seizures were followed by depression which lasted more than ten minutes, in the prolonged stimulation group and the control group, respectively.

4.3.5 The effects of some stressful conditions on epileptiform seizures in chickens.

The effects of various stressful treatments on birds preceding exposure to ILS at 14 fps are shown in Table 29.

There was no obvious effect of heat stress on seizure susceptibility. Birds which had been subjected to three minutes of heat stress without going into convulsions responded to ILS (14 fps) as well as those without the previous heat treatment.

The seizure susceptibility of day-old epileptic chicks was greatly affected by cold stress; the percentage of response in the treatment group was only 34.2 per cent compared to 89.5 per cent in the controls. The difference was highly significant ($P<0.01$). But the seizure susceptibility of birds tested at 34 and 54 days of age was not affected by cold treatment; the differences in frequencies of responses between the treatment and control groups at these ages were not statistically significant.

The seizure susceptibility of birds was not affected by previously subjecting the birds to ineffective ILS of 2 fps for ten minutes. The treated birds responded as well as the controls to ILS of 14 fps.

Epileptic chickens seemed to undergo great emotional disturbance when they were swung individually in a small wire-mesh cage. Birds were usually frightened by

the swinging motion and had difficulty keeping their balance. Some incidence of seizures, especially in day-old chicks, occurred during the swinging process. The percentage of birds which responded to ILS (14 fps) was considerably decreased in the treatment group compared to that in the controls. Nine of the day-old chicks which remained normal after the swinging treatment did not respond to the ILS that followed, and only 15.6 per cent and 35 per cent of the treated birds at 30 and 42 days of age, respectively, responded to ILS (14 fps) as compared to 84.4 and 100 per cent in the controls. The differences in frequency of birds responding to ILS (14 fps) between the treatment and control groups at each age were highly significant ($P<0.01$).

4.3.6 The response of epileptic chickens to successive intermittent light stimulations at various time intervals.

The time interval or resting period between two successive seizures was measured from the end of a seizure to the beginning of the next stimulation. The data on birds which responded to a second ILS after a previous seizure at one day, four weeks, and eight weeks of age at various time intervals are shown in Table 30. The time interval needed for day-old chicks to respond to a second ILS after a previous seizure was much shorter than for birds at older ages. Forty to 60 per cent of the day-old epileptic chicks which were subjected to ILS of 5, 14

and 20 fps responded to the second ILS and underwent a complete seizure without a resting period. If five minutes or more were allowed for the chicks to rest after a complete seizure, almost all of them convulsed when exposed to the second ILS.

At four and eight weeks of age birds did not respond to the second ILS if no resting period was allowed after a seizure, except for one bird tested with ILS of 5 fps at four weeks of age. All the birds tested with ILS of 14 fps and 20 fps were found to respond to the second ILS after a resting period of 30 minutes or more. But of those birds tested at 5 fps of ILS at four weeks of age, not more than 57 per cent of the birds responded to the second ILS even if 60 minutes of rest was allowed. At eight weeks of age all the birds responded to the second ILS of 5 fps after a rest period of 30 minutes or more. The results of analyses of the combined data of birds tested with ILS of 5, 14 and 20 fps at one day, four weeks and eight weeks of age showed that the responses of birds to the second ILS were significantly affected by the time interval or resting period between the seizures ($P<0.01$).

Table 31 shows the average seizure latencies and average seizure durations of first and second seizures of birds tested at one day, four weeks and eight weeks of age at various time intervals. The latency and duration

of the second seizure did not seem to be affected by the time interval between the two successive seizures. The results of analyses in Table 32 show that the difference between the latencies of the first and second seizures was not statistically significant ($P<0.05$) although the latency of the second seizures tended to be slightly longer than the first despite the time intervals between the two seizures. The difference between the durations of the first and the second seizures was not significant, except for birds tested at one day of age with a resting period of five minutes and birds tested at eight weeks of age with a 30-minute resting period (Table 32).

Tables 33 and 34 show the results of birds which were subjected to repeated ILS of 14 fps up to as many as eight trials at four and eight weeks of age. With a 15-minute interval between the tests, only three out of ten birds at four weeks of age responded to five successive stimulations, three stopped responding to ILS after the third seizure, one stopped after the second seizure, two stopped after the first seizure, and one bird did not respond to ILS at all; but if a 30-minute interval were allowed after a seizure, all five of the birds tested at four weeks of age were able to respond to each successive stimulation for all eight trials.

At eight weeks of age, ten birds were tested with repeated ILS at a 5-minute interval between the

tests; only one of the ten birds tested responded to six successive stimulations, one responded to five stimulations and four responded to two stimulations; two birds responded only to the first stimulation and two did not respond to ILS at all (Table 34). With a 15-minute interval between the tests, three of the five birds tested responded to all eight stimulations, one responded to seven stimulations, and one stopped responding after five stimulations.

The latency and duration of seizures fluctuated between the tests; however, the average latency of the first seizure tended to be shorter than those induced later.

4.4 Part III: Genetic studies on segregation of the *epi* gene and its effect on sex distribution, fertility, embryonic mortality and hatchability.

4.4.1 Segregation of the *epi* gene.

Crawford (1969, 1970) reported that epileptiform seizures in chickens are due to a single autosomal recessive gene, but he found a smaller number of epileptic chicks than expected from matings of epileptic or carrier males to carrier females (Crawford, 1970).

Table 35 shows the combined results of 12 hatches on *epi* gene segregation in chicks from different parental crosses. All chicks produced from matings of epileptic x epileptic were epileptic as shown by subjecting them to three successive exposures of ILS (14 fps) on hatching day. The numbers of epileptic and normal chickens obtained from

matings of epileptic male x carrier female and carrier male x epileptic female fit the expected 1 : 1 ratio. Those obtained from the matings of carrier x carrier also fit the expected 3 : 1 ratio. None of the chicks obtained from the matings between normal male and epileptic or normal females responded to ILS. The results of heterogeneity chi-square tests showed that no hatch effects existed in chicks from each cross.

4.4.2 The effect of the epi gene on sex distribution.

Table 36 shows the combined results of 12 hatches on sex distribution in chicks obtained from different crosses. The ratios of male and female epileptic and non-epileptic chicks from matings of epileptic x epileptic, carrier x carrier and normal x normal were 1 : 1. The ratios of male and female epileptic and carrier chicks from matings of epileptic male x carrier female, carrier male x epileptic female and normal male x epileptic female also fitted the expected 1 : 1 ratio. The results of the heterogeneity chi-square test showed that no hatch effects existed in chicks from each parental cross.

4.4.3 The effect of the epi gene on fertility, embryonic mortality and hatchability.

Table 37 shows the combined results of 12

hatches on fertility, embryonic mortality and hatchability of eggs obtained from different parental crosses.

There was no adverse effect on fertility due to the presence of the epi gene. The percentage of fertility ranged from 87 to 94 per cent among the parental crosses and the differences were not statistically significant.

The results of analyses showed that the differences in embryonic mortality and hatchability between fertile eggs obtained from various parental crosses were highly significant (Table 37); the hatchability percentages were high and the embryonic mortality percentages were low in fertile eggs obtained from matings of epileptic x epileptic and carrier male x epileptic female; the hatchability percentages were lower and the embryonic mortality percentages were higher in fertile eggs obtained from matings of epileptic male x carrier female, carrier x carrier, normal male x epileptic female, and normal x normal. The low hatchability and high embryonic mortality was mainly due to some of the hens laying many fertile eggs with poor shell quality. There were two hens in the epileptic male x carrier female group which laid 10 per cent of the fertile eggs and contributed 30 per cent of embryonic mortality in this group; three hens in the carrier x carrier group which laid 15 per cent of the fertile eggs and contributed 41 per cent of the embryonic mortality; one hen in the normal male x epileptic female

group which laid 44 per cent of the fertile eggs and contributed 57 per cent of the embryonic mortality; and one hen in the normal x normal group which laid 31 per cent of the fertile eggs and contributed 42 per cent of the embryonic mortality. The differences in hatchability and embryonic mortality between fertile eggs obtained from various parental matings were not statistically significant if the fertile eggs laid by these hens were removed from the data.

4.5 Part IV: Electroencephalographic (EEG) studies.

Typical tracings of resting EEG and EEG during ILS for epileptic, carrier and normal chickens are shown in Figure 7. Abnormal spiking patterns associated with the initial response of epileptic chickens to ILS of 5 to 20 fps are shown in Figure 8. Data on wave frequency and amplitude of the resting EEG and EEG during ILS of epileptic, carrier and normal chickens are summarized in Table 38 and the results of statistical analyses of these data are shown in Table 39.

4.5.1 Resting EEG.

The resting EEG of normal chickens had wave frequencies of 3.1 to 3.9 cps. The amplitude of waves was 38 to 40 μv (microvolts) recording from the electrodes which were located on the same side of the hemisphere or

intrahemispheric recording (leads A, B, C and D in Figure 5), and the amplitude was 56 to 114 μv from transhemispheric recording (leads E, F and G in Figure 5; Table 38).

The EEG pattern recorded from carriers was quite similar to that of normals. The frequency of waves was 2.8 to 3.9 cps and the amplitude recorded from leads A, B, C and D was 34 to 43 μv, and from leads E, F and G it was 61 to 116 μv.

The EEG of epileptic chickens differed from the EEG of carrier and normal chickens by having relatively slow waves with high amplitude. The frequency of the waves was 1.6 to 1.7 cps. The amplitude of waves recorded from leads A, B, C and D was 115 to 135 μv, and from leads E, F and G it was 150 to 194 μv.

The results of analyses showed that the wave frequency in the resting EEG was significantly lower and the wave amplitude was significantly higher in epileptic chickens than in carrier and normal chickens, but the wave frequency and amplitude were not different between carrier and normal chickens except that the frequency of brain waves was significantly higher in the normal chickens as recorded from leads A and B (Table 39).

4.5.2 EEG during intermittent light stimulation.

An abnormal EEG pattern was observed in epileptic chickens during ILS (Figures 7 and 8). It was associated

with spikes that were often increased in amplitude. The abnormal spikes often occurred shortly before the onset of severe clonic convulsive movements. They occurred at the time of the upwards and backwards or sideways movements of the head which were the initial response to ILS. Frequency of the abnormal spikes was found to be identical to the flash frequency of ILS. The amplitude of the spikes was 85 to 135 μv (Table 38).

No abnormal spiking was found in the EEG of carrier and normal chickens during ILS (Figure 7). The results of analyses showed that wave frequency and amplitude in the EEG of carrier and normal chickens were not affected by ILS (Table 39). No significant differences in wave frequency and amplitude in EEG during ILS were found between carrier and normal chickens except that the wave frequency recorded from lead A was significantly higher in normal chickens (Tables 38 and 39).

EEG tracings of epileptic chickens during seizures were not obtainable due to extensive artifacts which were correlated with violent convulsive movements. The brain waves returned to normal shortly after the end of the seizure.

5. DISCUSSION

5.1 Part I: Response of epileptic chickens to different types of stimulation.

Crawford (1969, 1970) reported that epileptiform seizures could be induced from epileptic chickens by strong visual and auditory stimulation, and by muscular fatigue. He found that convulsions could be easily induced from day-old chicks by beating a wire screen rhythmically over an opened chick box in which the chicks were kept, from older birds which were kept in cages by beating the cage wires, and by forcing individuals which were housed in floor pens to move around the pen for several minutes.

In the present study, it was found that convulsions could be induced by subjecting the epileptic chickens to heat, auditory and intermittent light stimulation.

5.1.1 Response of epileptic chickens to heat and cold stimulation.

No seizures were induced by subjecting epileptic chickens to cold stimulation, but some of the epileptic chickens underwent seizures during heat stimulation. This may be because the adaptation of chickens to the lower end of the temperature range is much better than to the higher end beyond the thermal neutral range, that is, the range of environmental temperatures in which the body temperature is not affected (Sturkie, 1965). Epileptic chickens were

found to be highly excited and disturbed during heat stress; this could be due to a painful sensation on their skin surface caused by heat stimulation, and response to rising body temperature. Birds crouched quietly during cold stimulation. Epileptic chickens might have obtained some protective effect in the cold temperature since reducing body temperature had been found to protect mice (Essman and Sudak, 1964; Fuller and Rappaport, 1952) and rats (Maier and Glaser, 1942) against convulsions. However, Lennox (1960) stated that in humans, neither extreme hypothermia of the body nor non-painful heat or cold application to the skin activated seizures.

5.1.2 Response of epileptic chickens to olfactory stimulation.

Olfactory stimulation is not a common precipitating factor in human epilepsy; Gowers (1901) reported one human epileptic patient who had seizures which were induced by an unpleasant smell, but no recent report was found on seizures which were induced by olfactory stimulation (Daube, 1965). Watson (1939, as quoted by Gruneberg, 1947) reported a species of deer mouse, Peromyscus rufinus, from which epileptic seizures could be induced only by means of olfactory stimulation.

No seizure was induced from epileptic chickens by olfactory stimulation using the smell of Indian incense

and dimethysulfide. Since the olfactory system in most avian species, including chickens, is not well developed except in birds in which smell may be important for locating food (Van Tienhoven, 1969), and chickens are not affected by odors in their surrounding environment (Sturkie, 1965), it is unlikely that olfactory stimulation would be one of the appropriate factors in causing seizures from epileptic chickens.

5.1.3 Response of epileptic chickens to auditory stimulation.

Audiogenic seizures were found in some species of deer mice (Chance and Yaxley, 1950; Dice, 1935; Summer, 1932; Watson, 1939, as quoted by Gruneberg, 1947), in house mice (Frings et al., 1951; Fuller et al., 1950; Fuller and Rappaport, 1952; Fuller and Sjuren, 1967; Witt and Hall, 1949) and in rats (Bayroff, 1940; Hall, 1947; Maier, 1939; Morgan and Galambos, 1942). Audiogenic seizures which were induced by a sudden loud noise have been reported in cattle (Atkeson et al., 1944), in goats (Hooper, 1916; Lush, 1930), in rabbits (Nachtsheim, 1939, 1940, 1941, as quoted by Gruneberg, 1947) and in humans (Foerster, 1931; Klove et al., 1965; Scott, 1969; Strobos, 1962); the startle effect had been considered as a significant component of these seizures.

Auditory stimulation was not very effective in inducing seizures from epileptic chickens. They mostly

responded to complex sound stimulation produced by banging a wire screen on top of the chick battery in which they were kept. The complex sound stimulus which was produced by banging a wire screen beside the chick battery was found relatively less effective in inducing seizures from epileptic chickens; only 5.6 per cent of the epileptic chicks at one day of age had seizures induced by this method as compared to 45.2 per cent induced by the former method. It seemed that complex sound stimulation alone was not the major factor in inducing seizures from epileptic chicks. The combined factors of complex sound stimulation, movement and vibration of the battery floor which created the excitement and emotional or startle effect on epileptic chickens was the important cause of inducing audiogenic seizures from these chickens.

Excitement or startle effect has been found to induce or intensify the symptoms of some nervous disorders which have been reported in poultry. Cole (1957) reported that the symptoms of congenital loco were more severe in affected poults which were excited by noise or vibration. The symptoms tended to be intensified in "congenital loco" quail (Sittman et al., 1965) and in "shaker" chickens (Scott et al., 1950) when the affected individuals were excited, and in "jittery" chickens (Godfrey et al., 1953) when the birds were frightened. Cole (1961) reported that paroxysmal symptoms in chickens could be induced by noise,

bright light, movement or whatever stimulation would produce a startle effect.

Gould and Morgan (1941, 1942) and Morgan and Gould (1941) reported that audiogenic seizures in rats could be induced by using a pure-tone stimulus produced from an electric oscillator. No seizures could be induced from epileptic chickens by subjecting them to pure-tone stimulation produced from an electric audiogenerator. The pure-tone stimulus created only slight startle or alertness in chickens at the very beginning of stimulation which did not seem to be enough to help in provoking epileptic response in the birds.

5.1.4 Response of epileptic chickens to photic stimulation.

Crawford (1969, 1970) reported that newly hatched epileptic chicks were susceptible to seizures when they were first removed from the darkened hatching compartment of the incubator and were exposed to sudden movement, noise and bright light. The present study showed that bright light to which the epileptic chicks were first exposed was an important factor in causing epileptic chicks to undergo seizures. Less than one per cent of the epileptic chicks had seizures if they were removed from the darkened or dimly lighted hatching compartment of the incubator into a dark room compared to seizures in 79.5 per cent of the epileptic chicks which were removed from

the same hatching compartment into the same room with light. 58.4 per cent of the chicks which remained normal in the dark had seizures soon after the light of the room was turned on. Intermittent light stimulation (ILS) was found more effective for inducing seizures in epileptic chickens; a very high percentage of day-old chicks had seizures under intermittent light stimulation.

Epileptic seizures which were induced by ILS have been extensively reported in humans. As early as 125 A.D. Apuleius (as quoted by Lennox, 1960) described the use of a spinning potter's wheel to detect epilepsy in slaves. Seizures induced by ILS have been described in many scientific papers and textbooks (Bickford and Klass, 1969; Cobb, 1947; Schmidt and Wilder, 1968; Ward et al., 1969). Seizures might also be induced by moving an object or fingers between the eyes of affected individuals and a bright light (Chao, 1962; Hutchison et al., 1958; Robb, 1965; Schmidt and Wilder, 1968; Scott, 1969; Sherwood, 1962), when watching television (Charlton and Hoefer, 1964; Gastaut et al., 1963; Karlsson, 1959; Klapetek, 1959; Lange, 1961; Mawdsley, 1961; Pallis and Louis, 1961; Pantelakis et al., 1962), or by driving past a row of trees in bright daylight (Cobb, 1947; Daube, 1965; Scott, 1969; Sutherland and Tait, 1969; Whitty, 1960). Cobb (1947) and Bickford and Klass (1969) suggested that fluctuation of light was a significant factor in photogenic seizures.

Intermittent light stimulation was also found to be highly effective for inducing seizures in epileptic chickens at other ages except in birds tested at three to seven days of age. The incidence of seizures was relatively low in birds tested at three to seven days of age compared to those tested at one day of age, but increased rapidly in birds tested after seven days of age.

The decrease in incidence of seizures in epileptic chickens exposed to ILS at three to seven days of age was also found in birds subjected to complex sound stimulation (Table 4). However, the incidence of seizures induced by heat stimulation was higher in birds tested during the first week after hatching and somewhat lower in birds tested at 14 and 28 days of age.

The reason for decreasing susceptibility to audiogenic and photogenic seizures in epileptic chickens during three to seven days of age is not known. It could be due to the maturation processes of the immature brain of young chicks, their energy supply and nutritional condition during the first few days after hatching, or some unknown physiological factors during this age.

Evidence for continuous maturation of the central nervous system in chicks has been shown in studies of EEG patterns (Peters et al., 1965); developmental changes in the electrical output of the brain were found in growing chicks. In developing chicks the brain waves were slower,

less regular, and of smaller amplitude; but the frequency, amplitude and regularity of the brain waves increased progressively with increasing age. The changes in permeability of the blood-brain barrier reported by Wood (1970) also give good evidence of the continuous postnatal cerebral development of young chicks; the permeability of the blood-brain barrier decreased in developing chicks.

Evidence for continuous postnatal cerebral maturation has also been shown in humans (Scott, 1969), in dogs (Charles and Fuller, 1956; Fox, 1964, 1967; Pampiglione, 1961, 1961a), in rabbits (Bishop, 1950), in mice (Servit, 1962), and in rats (Crain, 1952; Pylkko and Woodbury, 1961).

In humans, the susceptibility to and the frequency and duration of epileptic seizures increases progressively during postnatal development, accompanied by a decrease of seizure latency; these reflect the changes in the neuronal excitibility and the increasing capacity of neuronal interaction due to the structural and functional development of the immature brain (Purpura, 1969). In epileptic chickens the susceptibility to audiogenic and photogenic seizures was relatively low at three to seven days of age, but increased rapidly with increasing age. The results of the experiments in Part II (Tables 15 and 20) showed that seizure duration was somewhat shorter and the seizure latency somewhat longer in birds at three to seven days of

age; the seizure duration increased and the seizure latency decreased gradually after seven days of age. However, in most cases seizure duration began to decrease noticeably after 26 weeks of age.

The nutrient supply in chicks during the first few days after hatching is obtained principally from egg yolk which is absorbed into the abdominal cavity around the 19th day of incubation (Card and Nesheim, 1966; Schaible, 1970). They learn to eat and drink in the first few days but usually eat very little during this period. This natural starving process may affect their susceptibility to seizures since fasting has been reported to suppress epileptic seizures in humans (Penfield and Erickson, 1941; Robb, 1965). Repeated convulsions were common in day-old epileptic chicks and this convulsive activity might use up large amounts of the energy resources in the body; this, together with the nourishment condition of the chicks in the first few days of life could affect the energy supply to the brain.

In epileptic chickens, heat stimulation seemed to be an extreme stress. Romijn (1954) and Wekstein and Zolman (1967) reported that chicks maintain their body temperature rather poorly and have a relatively narrow range of thermal neutrality in the first few days of age. In epileptic chicks tested during three to seven days of age, the rapid rise in body temperature during heat stress

might cause some physiological changes in the body which might lower the seizure threshold of the chicks although their susceptibility to seizures was otherwise low during this age.

Intermittent light stimulation was chosen as the stimulation method for the study of epileptic chickens because of its high effectiveness in inducing seizures from chickens at most ages as compared to auditory stimulation, and at one day and after seven days of age as compared to heat stimulation. Although a high incidence of seizures could be induced from epileptic chickens during the first week of age with heat stimulation, the viability of birds which had been subjected to heat stimulation was greatly depressed compared to those which had been subjected to auditory and intermittent light stimulation. Besides, the incidence of seizures induced by heat stimulation sharply decreased in birds tested at 14 and 28 days of age.

5.2 Part II: Studies on epileptiform seizures in chickens induced by intermittent light stimulation.

5.2.1 The effects of age and flash frequency of intermittent light stimulation on epileptiform seizures in chickens.

5.2.1.1 Seizure susceptibility and seizure severity.

It has been reported in mice (Dice, 1935; Fuller and Sjursen, 1967; Ginsburg and Huth, 1947), in rats (Finger, 1943; Maier and Glaser, 1942a), in rabbits

(Hohenboken and Nellhaus, 1970; Nachtsheim, 1939, 1940, 1941, as quoted by Gruneberg, 1947), and in cattle (Atkeson et al., 1944) that susceptibility to seizures is affected by age. Most of the animals had seizures more frequently early in their lives, and the frequency decreased with increasing age. McGrath (1960) reported that dogs had seizures most frequently between one and three years of age. The epilepsy in Keeshonds may not occur until the affected dogs reached four years of age (Burns and Fraser, 1966). In humans, seizures were ten times more common in infants than in adults (Robb, 1965); the onset of seizures began more frequently during the first 20 years of life (Brain and Walton, 1969; Lennox, 1946; Putnam, 1945). Metrakos and Metrakos (1966), from studies of abnormal EEG patterns of centrencephalic epilepsy in humans found that penetrance of the dominant gene which was responsible for the abnormality was very low at birth, but increased gradually to almost complete penetrance between 4½ and 16½ years of age, and declined to almost no penetrance after 40 years of age.

The susceptibility to photogenic seizures in epileptic chickens was also found to be significantly affected by their age. Seizure susceptibility was very high at day-old, decreased sharply during three to seven days of age, and rapidly increased after two weeks of age. The incidence of seizures was as high or higher in birds

tested at or after eight weeks of age (except in birds which were subjected to ILS with frequency lower than 10 fps) than that in birds tested at one day of age. The increase in incidence of seizures with age was relatively slower in birds subjected to ILS with frequencies lower than 10 fps as compared to those subjected to ILS with frequencies above 10 fps.

As mentioned before seizure susceptibility might decrease with age in animals. However, it increased in epileptic chickens as the birds grew older. In many instances, seizure susceptibility in adult chickens reached 100 per cent. The incidence of seizures was also high in day-old epileptic chicks and in birds tested after one week of age, but the incidence of seizures was low in young rats (Gruneberg, 1947; Finger, 1943) and seizures were usually not found in very young animals such as in cattle (Atkeson et al., 1944), rabbits (Nellhaus, 1970; Nachtsheim, 1939, 1940, 1941, as quoted by Gruneberg, 1947), and in mice (Swinyard et al., 1963; Vicari, 1950; Witt and Hall, 1949).

The degree of abnormal symptoms of some nervous disorders in poultry was found to be affected by age. The symptoms of tremor in chickens (Hutt and Child, 1934), congenital loco in quail (Sittmann et al., 1965) and vibrator in turkeys (Kulenkamp et al., 1968) were found to decrease with age. Savage and Collins (1972) reported

that star-gazing in quail was detected occasionally between hatching and three weeks of age but the symptoms tended to become more pronounced in older birds.

In baboons (Papio papio), age did not seem to affect the seizures induced by ILS (Killam et al., 1967). The severity of audiogenic seizures in rabbits (Hohenboken and Nellhaus, 1970; Nellhaus, 1963) and in deer mice (Dice, 1935; Watson, 1939, as quoted by Gruneberg, 1947) tended to decrease with increasing age.

In epileptic chickens, severe and prolonged seizures were found more often in day-old chicks than in older birds; about 31 per cent of seizures induced from day-old chicks lasted longer than 120 seconds as compared to no prolonged seizures in birds tested at three to seven days of age and not more than five per cent in birds tested after seven days of age.

A short period of unconsciousness or coma following a seizure has been described in humans (Gastaut, 1954; Gowers, 1901; Scott, 1969; Sutherland and Tait, 1969; Thorpe et al., 1961; and others), in cattle (Atkeson et al., 1944), and in rats (Golub and Morgan, 1945; Griffiths, 1942a; Hamilton, 1942). In epileptic chickens, coma following a seizure was not common and was found mostly in day-old chicks. In about five per cent of the seizures produced in day-old chicks coma followed; whereas coma followed in less than two per cent of the birds tested

between three and eight weeks of age, and no coma was observed in birds tested at older ages.

In epileptic chickens, the incidence of complete seizures was also significantly affected by age. It was found to follow the same pattern as the seizure susceptibility; the incidence was high at one day, decreased sharply during three to seven days, and increased rapidly after seven days of age; by 52 weeks of age almost all the chickens which responded to ILS had complete seizures except that five per cent of the birds which were subjected to ILS of 8 fps had incomplete seizures.

Incomplete seizures have been described in rabbits (Gruneberg, 1947; Nellhaus, 1963), in deer mice (Frings et al., 1951; Gruneberg, 1947), in house mice (Frings et al., 1951; Witt and Hall, 1949), and in rats (Gruneberg, 1947). In rabbits, incomplete seizures occurred more often in animals of advanced age (Gruneberg, 1947). Incomplete seizures were also found to occur in epileptic chickens which were subjected to ILS. They occurred more often in epileptic chickens tested between three days and 26 weeks of age than in birds tested at one day and after 26 weeks of age.

5.2.1.2 Seizure latency.

Antonitis et al. (1954) found that the average latency of audiogenic seizures in rabbits was highly variable

in strains selected for seizure susceptibility; the average seizure latency was much shorter in a high susceptibility strain (6.8 seconds) as compared to the control (17.0 seconds). Frings and Frings (1952, 1953) also found that the latency in strains of mice selected for high seizure susceptibility was about six seconds as compared to the latency of the original unselected mice with average latency of audiogenic seizures of about 27 seconds. In the brown rats described by Morgan (1941) seizure latency was much longer (40-60 seconds) than that found by Smith (1941) in his white rats (11.2 seconds). However, the average latency found in rabbits (Antonitis et al., 1954; Nellhaus, 1963), in mice (Frings et al., 1951; Frings and Frings, 1952, 1953; Witt and Hall, 1949), and in rats (Morgan, 1941; Smith, 1941) was generally less than 60 seconds which was similar to that found in epileptic chickens in the present study. From observation of 2490 seizures, it was found that 99.4 per cent of them had latencies of 60 seconds or less and in 80.7 per cent of them latencies were not longer than 30 seconds.

The relationship between age and seizure latency in the animal species mentioned above has not been reported. In epileptic chickens the seizure latency of birds tested at various ILS frequencies was significantly affected by age. The average seizure latencies obtained from birds tested at three to seven days of age were longer than in

birds tested at one day of age, and then they decreased with increasing age.

5.2.1.3 Seizure duration.

In humans, the duration of seizures depends upon type and severity of the disorder; seizures may last for only a few seconds, or may last for several minutes or more (Kooi, 1971; Lennox, 1946; Putnam, 1945; Robb, 1965; Schmidt and Wilder, 1968; Tucker and Forster, 1950; Ward et al., 1969; Wishik, 1958).

In animals such as goats (Lush, 1930), rabbits (Nachtsheim, 1939, 1940, 1941, as quoted by Gruneberg, 1947; Nellhaus, 1963), deer mice (Gruneberg, 1947), and house mice (Frings et al., 1951), seizures generally lasted less than 60 seconds. McGrath (1960) reported that the tonic-clonic type seizures in dogs lasted from 30 seconds to 180 seconds. Auer and Smith (1940) reported that the tonic convulsive phase of audiogenic seizures in rats lasted from five to 43 seconds and the clonic convulsive phase lasted from 70 to 330 seconds. Antonitis et al. (1954) found that the duration of audiogenic seizures in rabbits could be varied by selection; the duration of seizures was 79.3 seconds in a high susceptibility strain and was 22.6 seconds in the control strain.

Crawford (1969, 1970) reported that the duration of audiogenic seizures in epileptic chickens varied within

individual birds; seizures might last for only a few seconds in mild cases, or might last up to 30 minutes or more in severe seizures. In the present study the duration of complete seizures induced with ILS ranged from 12 seconds to 90 minutes.

The results of analyses showed that the seizure duration of epileptic chickens was significantly affected by age except in birds tested at 12 and 40 fps of ILS (Table 16). The average seizure durations obtained from birds tested at one day of age was usually much longer than in birds tested at other ages; it tended to be short in birds tested at three to seven days of age, and tended to shorten again in birds tested at or after 52 weeks of age.

Status epilepticus (grand mal seizures occurring in succession) and petit mal status (petit mal seizures occurring in succession) have been described in humans (Putnam, 1945; Robb, 1965; Scott, 1969; Tucker and Forster, 1950; Wishik, 1958; and others). Nellhaus (1963) found that status epilepticus also occurred in rabbits, especially in young individuals. Hamilton (1942) reported that one of his rats had repeated convulsions lasting for more than 90 minutes. In epileptic chickens, severe prolonged seizures (lasting longer than 120 seconds) which were somewhat similar to status epilepticus in humans were found mostly in birds tested at one day of age (30.5 per cent) and less commonly in birds tested between two and 104

weeks of age (0.5 to 5.1 per cent); no prolonged seizures were found in birds tested during three to seven days of age.

The most effective frequency of ILS for inducing seizures from light sensitive human epileptic patients ranges from 10 to 15 fps (Bickford and Klass, 1969; Daube, 1965; Sherwood, 1962), but it varies from individual to individual and from time to time (Daube, 1965). In baboons (Papio papio) the effective frequency of ILS ranges from 20 to 30 fps, and the most effective frequency is 25 fps (Fischer-Williams et al., 1968; Killam et al., 1967; Naquet et al., 1968). In epileptic chickens the ILS frequencies of 2 to 40 fps were effective for inducing seizures from the birds, except that ILS of 2 fps was no longer effective in birds after one day of age. The most effective ILS frequency for inducing seizures from epileptic chickens ranged from 10 to 20 fps. But in birds tested at 52 and 104 weeks of age, ILS of 5, 8, 30 and 40 fps were also highly effective. Birds tested with lower ILS frequencies, especially with a frequency of 5 fps, tended to have longer seizure latency than birds tested with higher ILS frequencies (Table 15).

Seizure duration seemed to be unaffected by the flash frequency of ILS; the average seizure durations obtained from birds tested at various ILS frequencies at most ages were not significantly different except in birds

tested at one day and at four, six and seven weeks of age (Table 17).

5.2.2 The effect of sex on epileptiform seizures in chickens.

Lennox (1946) stated that in humans the incidence of seizures was about the same in both sexes; females began to have seizures earlier than males; the peak age for the onset of seizures in females was in the 13th year, and in males it was in the 14th year; he suggested that the early onset in females might be due to a greater hereditary tendency in females and the earlier maturation of females than males. Gowers (1901) reported that the incidence with which the two sexes had seizures varied considerably in different periods of life; the incidence of seizures in females was greatly in excess of that in males at infancy; during the later period of childhood the numbers of both sexes suffering from seizures were nearly equal; at puberty the incidence in females was higher and it declined thereafter so that by middle age incidence was higher in males.

Farris and Yeakel (1942, 1942a) found that in rats the incidence of seizures in males and females changed with age; at 26 to 40 days of age male rats reacted more often than females, but the incidence of seizures in males reduced sharply around 125 days or more

of age, and the incidence of seizures was down to about half the incidence of seizures in females at 240 days of age; the seizure susceptibility in both sexes decreased considerably in advanced age. However, Maier and Glaser (1942a) did not find sex to be related to seizure susceptibility in rats. Woolley and Timiras (1962) found that susceptibility to seizures in rats induced by electric shock was affected by the female sex hormone; by administration of estradiol to intact mature males and to ovariectomized immature and mature females the threshold of seizures was markedly reduced.

Sex did not seem to have an effect on the response of baboons to ILS (Killam et al., 1967), nor did it affect incidence of audiogenic seizures in rabbits (Nellhaus, 1963) and in deer mice (Dice, 1935).

In epileptic chickens no obvious differences were between males and females in seizure susceptibility and seizure severity; the changes in seizure susceptibility and incidence of complete seizures with age closely followed each other in male and female chickens.

Seizure latency was not found to be different between male and female chickens; however, the average seizure duration tended to be longer in males than in females. This might be because the incidence of severe seizures with prolonged duration was higher in males than in females; statistical analyses showed that the incidence

of severe seizures with durations longer than 900 seconds was significantly higher in males than in females (Table 24).

Penfield and Erickson (1949, as quoted by Novakova, 1963) suggested that "Blocking of an epileptic seizure occurs as a consequence of a disturbance of the equilibrium between the increased consumption of energy-providing substances during an epileptic fit and the possibility of fully supplying the nervous tissue by the blood circulation". This means that the maintenance of an epileptic seizure is related to supplying of the various needs of the central nervous system during epileptic activity. Killer and Brody (1944, as quoted by Sturkie, 1965) and Mitchell and Haines (1927, as quoted by Sturkie, 1965) found that metabolic rate (expressed in relation to surface area) in male chickens was 5.7 to 13.0 per cent higher than in females, depending on the breed. Thus it might be possible that male epileptic chickens were somewhat more vigorous and energetic and therefore tended to have severe prolonged seizures of longer duration than females.

5.2.3 The effect of parentage on epileptiform seizures in chickens.

Epileptic chickens which were obtained from various matings did not respond consistently to ILS at

frequencies of 5, 14 and 20 fps. However, the highest percentages of seizure susceptibility and complete seizures and the shortest average seizure latency were always obtained from the progeny of epilepsy carrier sires; the lowest percentages of seizure susceptibility and complete seizures and the longest average seizure latency were always obtained from the progeny of epileptic sires.

Crawford (1970) reported that there was a deficiency of epileptic offspring produced from families of heterozygous dams (epileptic male x carrier female and carrier male x carrier female). In the present study, the results showed that epileptic birds produced from heterozygous sires seemed to be more susceptible to ILS than epileptic birds produced from epileptic sires, irrespective of the genotype of their dams. The method of auditory stimulation used in the present study was similar to that used by Crawford (1969, 1970). It was found that this method was not very effective for inducing seizures from epileptic chickens in the present study as compared to intermittent light stimulation (Tables 4, 5, 6, 8 and 9). It might be expected that some of the less-susceptible birds which were subjected to auditory stimulation would escape detection by not showing a seizure in the first few days of life. Assuming that the matter of less-susceptible birds escaping detection by auditory

stimulation was due to chance, then the deficiency in epileptic offspring of heterozygous dams found in Crawford's experiments (1970) might be due to less-susceptible epileptic chicks of heterozygous dams being more frequent in number than those of epileptic dams. In the present study, epileptic chickens were identified by observing a spontaneous seizure or a seizure induced by auditory stimulation before being used in the experiment, except for progeny of epileptic x epileptic. It is possible that most of the epileptic birds which were identified by these methods were more susceptible to seizures; it could be due to chance that these highly-susceptible epileptic birds were more frequent in number among the epileptic progeny of heterozygous sires than among the epileptic progeny of epileptic sires. If this occurred in the present study, then it would be reasonable to expect that the percentages of seizure susceptibility and incidence of complete seizures obtained from epileptic progeny of heterozygous sires would be higher and the average seizure latency would be shorter than epileptic progeny of epileptic sires.

5.2.4 The effect of prolonged intermittent light stimulation on epileptiform seizures in chickens.

Prolonged ILS after the initiation of a seizure had no significant effect on the duration of that seizure. Thus ILS was only an important factor in inducing seizures

from epileptic chickens, but had little or no effect in maintaining or prolonging the duration of seizures once they were initiated. However, the frequency of post-seizure depression was obviously increased in birds subjected to prolonged ILS; about 93 per cent of the group with prolonged stimulation had seizures followed by post-seizure depression compared to about 42 per cent of those in the control group. No reports of the effects of prolonged ILS on epileptic seizures in animals and in humans have been made.

Cole (1961) found that after an attack, "paroxysm" chickens relaxed and rested quietly for varying periods of time, depending upon the degree of stimulation. In epileptic chickens, birds might recover immediately after the seizure or they might have a depression following the seizure requiring several seconds to more than 30 minutes to recover. Variation in recovery periods are reported for rabbits (Antonitis et al., 1954; Nellhaus, 1963), deer mice (Dice, 1935; Gruneberg, 1947), and house mice (Frings et al., 1951; Hamilton, 1942). Antonitis et al. (1954) and Nellhaus (1963) reported that affected rabbits might recover immediately after a seizure, or the animals might remain motionless and remain limp for up to 30 minutes before complete recovery. This is similar to the situation found in chickens. Antonitis et al. (1954) found that the recovery period was much longer in a

seizure-susceptible strain of rabbits than in the control. Dice (1935) and Gruneberg (1947) reported that in affected deer mice sluggishness and relaxation might follow a seizure. It usually took one or two minutes for deer mice to recover from the seizure (Dice, 1935), but in some animals the recovery period might take up to 25 minutes (Gruneberg, 1947). Frings et al. (1951) reported that the recovery time in house mice was 30 to 50 seconds with a range of 10 to 200 seconds depending on the severity of the seizure. Griffiths (1942a) reported that some rats remained entirely motionless following a seizure. Chance (1957) found that spontaneous activity of rats after a seizure was virtually abolished for two to 15 minutes or more and the animal did not resist handling during this period. Hamilton (1942) reported that after a seizure rats sat quietly in a corner for a few seconds to as long as 30 minutes before they resumed their normal exploratory cage behavior.

In human epilepsy, a long phase of generalized depression usually follows the termination of a grand mal seizure; there is a very short period of complete unconsciousness at first, followed by a period of post-seizure effects varying from stupor or regressive mental confusion to paralysis varying from less than a minute to hours or days (Gastaut, 1954; Gowers, 1901; Lennox, 1946, 1960; Novakova, 1963; Penfield and Erickson, 1941; Robb,

1965; Scott, 1969; Sutherland and Tait, 1969; Todd, 1855, as quoted by Meyer and Portnoy, 1959; and others). Novakova (1963) called this depression "postparoxysmal inhibition". Kreindler (1955, as quoted by Novakova, 1963) and Krushinsky (1949, as quoted by Novakova, 1963) suggested that the nature of the postparoxysmal inhibition was a supraliminal inhibition which protected the nerve cells against complete exhaustion. Todd (1855, as quoted by Meyer and Portnoy, 1959) and Robertson (1869, as quoted by Meyer and Portnoy, 1959) suggested that the postseizure paralysis was due to portions of the brain which were most involved by the discharge. Using EEG recording from the post-epileptic stage of grand mal seizures, Gastaut (1954) provided evidence for exhaustion of neuronal systems in which epileptic discharges developed; the EEG showed a period of complete electrical silence following a seizure and then a period of very slow waves. In epileptic chickens, it could also be possible that the postseizure depression behavior was due to the exhaustion of neuronal systems in the brain caused by the convulsive activity of the birds; the duration of depression might be related to the degree of exhaustion and the speed of the recovering process in the brain after the seizure.

5.2.5 The effects of some stressful conditions on epileptiform seizures in chickens.

The results of experiments in Part I (section

4.2.1) showed that seizures could be induced in epileptic chickens by heat stimulation. In this experiment the seizure susceptibility of birds which remained normal after heat stress did not seem to be affected by the heat treatment since both the treated and control birds responded to ILS (14 fps) (Table 29).

No seizures were induced in epileptic chickens during cold stimulation. Seizure susceptibility diminished significantly in day-old chicks which had been subjected to acute cold stress prior to ILS (14 fps). But the seizure susceptibility of older birds did not seem to be affected by the cold stress. This may be because the chick has poor thermoregulating ability in the first few days of life (Romijn, 1954; Wekstein and Zolman, 1967, 1971). Whittow (1965) suggested that the increase in ability to adapt to cold environmental temperatures in older birds is correlated with increase in insulation and rate of metabolic heat production and with decrease in the ratio of surface to body mass. A decrease in incidence of audiogenic seizures due to the reduction of body temperature has also been reported in mice (Essman and Sudak, 1964; Fuller and Rappaport, 1952) and in rats (Maier and Glaser, 1942); the lower the body temperature, the greater the protective effect that was achieved in these animals.

Maier and Glaser (1942) reported that the incidence of audiogenic seizures in rats was greatly

reduced by previously subjecting the rats to ineffective auditory stimulation; they suggested that it could be due to sensory adaptation of the animals in that the first ineffective sound stimulus made the ear less responsive to certain pitches of the effective sound stimulus which were essential for producing the seizure. Adaptation to auditory stimulation was also reported by Maier and Glaser (1940a); they found that rats which were tested once a day for a period of time had formed some adjustment which inhibited the animals from producing seizures. In this experiment, the seizure susceptibility in epileptic chickens was not affected by previously subjecting the birds to ineffective ILS of 2 fps; the treated birds responded as well as the controls to the effective ILS of 14 fps. Previously exposing epileptic birds to ineffective ILS of 2 fps did not seem to cause the birds to make any kind of adaptation which would inhibit them from responding to effective ILS of 14 fps.

There is a definite correlation between seizures and emotion in many human epileptics (Lennox, 1946, 1960); many patients will have a seizure immediately after some unpleasant or terrifying experience, or may have an increase of seizures during periods of unhappiness or worry. Humphrey and Marcuse (1939) reported that emotional disturbance created by swinging rats in a cage during auditory stimulation tended to increase the seizure susceptibility

of the animals. However, Hamilton (1942) reported that in rats extremely intense stimulation such as swinging the animal by his tail, or tossing the animal to a table several feet away, tended to inhibit seizures. In the present study, epileptic chickens were obviously disturbed and excited during the swinging process in the cage. Some birds, especially day-old chicks, had seizures inside the cage during the swinging treatment. However, in birds which remained normal during the swinging treatment, the seizure susceptibility was significantly decreased as compared to that of the controls.

The loss in ability to respond to ILS (14 fps) in birds immediately after the cold or swinging treatment was only temporary; most of these birds regained their ability after a two-hour period of rest.

5.2.6 The response of epileptic chickens to successive intermittent light stimulations at various time intervals.

Crawford (1969, 1970) reported that after a seizure, an affected bird would not undergo another seizure for several hours. In the present study, it was found that the response of birds to successive ILS of 5, 14 and 20 fps after a previous seizure was significantly affected by time intervals or resting periods after the first seizure. Successive seizures could be induced in most birds after a period of 15 minutes or 30 minutes of

recuperation; the time required for recuperation was even shorter if the birds were tested at one day of age. Forty to 60 per cent of the day-old chicks required no resting interval to produce a seizure right after a previous one. Almost no birds tested at four and eight weeks of age responded to the second ILS. A five-minute interval seemed to be long enough for almost all of the day-old chicks to respond again to a second ILS.

The less effective ILS frequency of 5 fps did not seem to affect the response of birds tested at one day and eight weeks of age to a second stimulation if the resting period after the first seizure was long enough. However, in birds tested at four weeks of age only 57 per cent of the birds responded to a second ILS of 5 fps even though the birds were allowed to have a 60-minute period of rest after the first seizure.

Lush (1930) reported that a second seizure could not be induced from "nervous" goats until the affected animals rested for 20 to 30 minutes after the first seizure. Cole (1961) reported that paroxysmal attacks in chickens occurred at irregular intervals, but could be induced only 30 minutes or longer after a previous attack.

Stone (1957) reported that the acetylcholine content of the dog brain decreased in pentylenetetrazol-induced convulsions. Richter and Crossland (1949) also reported that the acetylcholine content of the rat brain was

reduced in electrogenic seizures by as much as 56 per cent; they found that convulsions could not be induced until the acetylcholine level had returned to the normal level after the initial fall due to electrical stimulation. Hyde et al. (1949) found that convulsive activity in the cat brain can be restarted after it had come to an end by the direct application of acetylcholine to the cerebral cortex.

In the present study a period of rest after a seizure seemed to be necessary, especially for older chickens, before the next convulsion could be induced. It is possible that epileptic chickens also used this resting period to restore the acetylcholine level which was decreased after a seizure. Thus duration of the resting period needed for a bird to regain the ability to respond and undergo seizure at a successive ILS would be related to the rate of restoration of acetylcholine in the brain of the bird.

Woodbury et al. (1957) found that the recovery process which restores normal functioning of the brain following a seizure requires energy, and that the main source of energy is from blood glucose, since the glycogen content of the brain is usually small. In general, only water, CO_2 and O_2 cross the cerebral capillaries with ease; the exchange of other substances is low (Ganong, 1969). It may be possible that the blood glucose and some substances which are necessary for restoring the normal function of

the brain after a seizure could more rapidly enter the brain cells; also the acidic metabolic products which accumulate in the cerebral tissues during seizures and which have to be relieved before normal cerebral function could be resumed, could be removed much faster in day-old chicks than in birds at four and eight weeks of age, since Wood (1970) reported that the blood-brain barrier is more permeable in younger chicks than in older birds. If this is true, it may account for the reason that older birds need longer time intervals to regain their ability to respond to successive ILS after a previous seizure than do day-old chicks.

The latency and duration of the second seizure did not seem to be affected by the first seizure and time intervals between seizures. The average latency and average duration of the second seizure induced by ILS at different time intervals were usually not significantly different from those of the first seizure.

A series of seizures could be induced from epileptic chickens if the time interval was long enough for the birds to recover from a previous seizure. Although it varied from individual to individual, a 30-minute interval seemed to be long enough for most of the birds at four weeks of age and a 15-minute interval seemed to be long enough for most of the birds at eight weeks of age to respond to a series of five to eight intermittent light

stimulations.

5.3 Part III: Genetic studies on segregation of the *epi* gene and its effect on sex distribution, fertility, embryonic mortality and hatchability.

5.3.1 Segregation of the *epi* gene.

Crawford (1970) reported that the *epi* gene which caused epileptiform seizures in chickens had incomplete penetrance. Two individuals obtained from matings of epileptic males and epileptic females did not exhibit seizures and the numbers of epileptic chicks which were obtained from matings of epileptic male x carrier female and carrier x carrier were lower than expected (Crawford, 1970). In the present study the results showed that the penetrance of the *epi* gene was complete by using ILS for seizure induction. All of the 218 epileptic chicks from matings of epileptic males and females exhibited seizures which were induced by ILS of 14 fps in three tests at hatching day. The number of epileptic chicks from matings of epileptic male x carrier female was only insignificantly lower than expected. The numbers of epileptic chicks obtained from matings of carrier male x epileptic female and carrier x carrier were very close to the expected numbers.

The difference between Crawford's (1970) findings that a deficiency of epileptic offspring produced from matings of epileptic male x carrier female and carrier

x carrier, and those of the present study could be explained if it is assumed that ILS used in the present study was more effective than the auditory stimulation used by Crawford. By using an effective frequency of ILS, more or all of the less susceptible epileptic chickens were detected at hatching day in the present study. However, to explain Crawford's finding, it should be assumed that auditory stimulation was not highly effective for inducing seizures from epileptic chickens, especially in the less susceptible individuals; thus, by chance, some of the less susceptible birds which were produced by matings of carrier females to epileptic and carrier males failed to respond to auditory stimulation in their first few days of life and therefore were grouped as normal chickens. This was probably the cause of distortion of the ratios of progeny of heterozygous dams from the expected in matings of epileptic male x carrier female and carrier x carrier.

5.3.2 The effect of the epi gene on sex distribution.

Lennox (1946) reported that the incidence of seizures in humans was about the same in both sexes. However the proportion of males and females suffering from epileptic seizures varied at different ages (Gowers, 1901; Lennox, 1946). The epi gene in chickens did not seem to affect sex distribution; the frequencies of male and female epileptic and carrier chicks tested at hatching day all

fitted the expected 1 : 1 ratio.

5.3.3 The effect of the epi gene on fertility, embryonic mortality and hatchability.

Cole (1961) reported that after several attacks the "paroxysm" chickens showed a syndrome of poor growth, and all of the affected birds died by 14 weeks of age, but hatchability and early growth rate were not affected. Kulenkamp et al. (1968) reported that the vi (vibrator) gene had no adverse effect on fertility, hatchability and growth rate of the affected turkeys except that mortality was higher in affected birds than in normals. Crawford (1969, 1970) reported that the epi gene did not affect growth rate, livability and fertility in chickens.

In the present study, fertility was high in all six types of matings which involved epileptic, carrier and normal chickens. The epi gene did not affect the fertility of birds which carried it. The epi gene did not seem to affect hatchability and embryonic mortality. The hatchability of epileptic chicks from matings of epileptic x epileptic was high (Table 37). The numbers of epileptic chicks produced from matings of epileptic male x carrier female, carrier male x epileptic female and carrier x carrier, and the numbers of carrier chicks produced from matings of epileptic male x carrier female and carrier male x epileptic female were not significantly different from

the expected numbers (Table 35). Although there were significant differences found in hatchability and embryonic mortality between different matings, these differences were apparently caused by some hens which produced eggs with poor shell quality. No significant differences were found if data from the hens which produced poor-shelled eggs were deleted (Table 37).

5.4 Part IV: Electroencephalographic (EEG) studies.

5.4.1 Resting EEG.

In human epilepsy, a normal EEG is usually found in the intervals between seizures (Gastaut, 1954); however, abnormal patterns such as polyspikes-and-waves, spikes-and-waves, and other abnormalities of rhythm have been recorded in the inter-seizure EEG (Gastaut, 1954; Lennox, 1946, 1960; Metrakos and Metrakos, 1961, 1961a, 1966; Penfield and Erickson, 1941; Ward et al., 1969; Wilder, 1968). Abnormal EEG found in the intervals between seizures are usually not specifically related to the type of seizure except for the spike-and-wave pattern of petit mal seizures, and the spiking in the anterior temporal region during light sleep of persons with psychomotor seizures (Lennox, 1960). Lennox (1946) reported that in some patients grand mal seizures could be predicted many hours in advance by the increasing frequency of abnormal waves.

In animals, Burns and Fraser (1966) reported that epilepsy in Keeshonds may not occur until the dogs reach four years of age; but the affected animals can be detected by showing an abnormal EEG at about one year of age (Croft and Stockman, 1964; Croft, 1968). Nellhaus (1963) reported that seizure-susceptible rabbits had a normal EEG with a fairly regular wave frequency of 7 cycles per second (cps) in the intervals between seizures, but that spontanous spikes were found in the EEG of highly seizure-susceptible animals.

The resting EEG pattern of epileptic chickens was found to differ from that obtained from carrier and normal chickens. The resting EEG of epileptic chickens was characterized by slow waves with relatively high amplitude as compared to the resting EEG patterns of carrier and normal chickens which were characterized by faster frequency with lower amplitude. The differences were found to be statistically significant. Carrier and normal chickens did not differ in wave frequency and amplitude in resting EEG except that the resting EEG recorded from leads A and B had faster wave frequency in normal chickens than in carriers.

Comparing the average frequency of waves recorded from leads A and B between normal, carrier and epileptic chickens, it was found that in carrier chickens the resting EEG was about 1.0 cps slower than in normal

chickens, and in epileptic chickens the resting EEG was about 2.0 cps slower than in normal chickens. This is coincident with single and double doses of the epi gene in carrier and epileptic chickens.

The average wave frequencies in resting EEGs recorded at different leads were similar or close to each other in epileptic, carrier and normal chickens, and the variation in wave frequency among individuals was small as shown by the frequency ranges (Table 38). The wave amplitudes of the transhemispheric EEG were somewhat larger than that of the intrahemispheric records in epileptic, carrier and normal chickens. Klemm (1969) suggested that this type of phenomenon might be due to less synchrony between transhemispheric cortical generators, resulting in greater interelectrode voltage difference and less voltage cancellation. The variation of wave amplitudes within epileptic chickens, as shown by amplitude ranges, was much larger than that within carrier or normal chickens.

5.4.2 EEG during intermittent light stimulation.

Bickford et al. (1953), Stevens (1962), Walter and Walter (1949), and many other workers found that human epileptics usually show abnormal high-voltage spiking patterns during ILS. Polyspikes and polyspike-and-wave complexes with large amplitude were also found in baboons during ILS (Killam et al., 1966, 1967, 1967a, 1967b;

Naquet, 1969; Naquet et al., 1968, 1969). Abnormal spikes with high amplitudes were found in the EEG of epileptic chickens during ILS. The spiking waves usually occurred shortly before the onset of the severe clonic convulsive movements and corresponded to the initial response of the birds to ILS.

The frequency of abnormal spikes was found to be identical to the flash frequency of ILS. A similar finding has been reported in the EEG of human epileptics (Walter and Walter, 1949) and in the EEG of baboons (Naquet, 1969). No abnormal spiking was found in the EEG of carrier and normal chickens during ILS.

The wave frequency and amplitude in the EEG during ILS did not differ between carrier and normal chickens, except that the EEG recorded from lead A had a faster wave frequency in normal chickens than in carriers.

6. SUMMARY AND CONCLUSIONS

The present studies are an attempt to obtain further information on the behavioral and physiological bases of genetic epileptiform seizures in chickens, caused by the autosomal recessive gene *epi*.

The stock used in these studies was obtained from a mongrel population based on crosses of several breeds with the Fayoumi, in which the gene was first discovered (Crawford, 1969, 1970). Birds used in these studies were obtained from three successive generations produced from this mongrel population. A total of 2025 birds, which consisted of 1603 epileptic, 120 carrier, 105 normal, and 197 non-epileptic birds with unknown genotype (*Epi*-) were used. They were obtained from 48 hatches during June, 1969 to December, 1971. Some individuals were used in more than one experiment. The birds were tested between one day and 104 weeks of age.

All birds were reared in heated chick battery brooders from one day to four weeks of age, and were kept in a larger unheated rearing battery from four to 12 or 16 weeks of age. They were moved from the rearing battery to floor pens between 12 and 16 weeks of age, and were then moved from the floor pens to individual cages at about 26 weeks of age. Feed and water were supplied *ad libitum*. Birds were kept under a 24-hour artificial light regime and

were fed a commercial chick starter ration from hatching to four weeks of age. They were kept under a 14-hour artificial light regime after four weeks of age. They were fed a commercial chicken grower ration from four to about 26 weeks of age and then were fed a commercial layer ration after 26 weeks of age.

In Part I of these studies, a suitable method for inducing seizures was sought. Birds were subjected to heat, cold, olfactory, auditory and photic stimulation. Seizures occurred in newly hatched epileptic chicks when they were first exposed to bright light, and could be induced thereafter by heat, complex sound and intermittent light stimulation (ILS). Cold, olfactory and pure-tone auditory stimulation were found to be ineffective for inducing seizures in epileptic chickens. The most satisfactory stimulus was ILS.

In Part II, ILS was used as the stimulus in studying the effects of external and internal environmental factors on seizures in epileptic chickens.

Epileptiform seizures in chickens were affected by age and by the flash frequency of ILS. Seizure susceptibility and incidence of complete seizures were relatively high at one day of age, decreased sharply during three to seven days of age, and increased rapidly again at and after two weeks of age. Incomplete seizures occurred most often in birds tested between three days and

26 weeks of age. Coma after seizures was not common and occurred mostly in day-old chicks. Severe prolonged seizures occurred mostly in day-old chicks and were not common at other ages. The frequency of seizures having short duration increased gradually with age. The seizure latency tended to be longer in birds tested at three to seven days of age as compared to that in birds tested at one day of age, but decreased gradually in length with increasing age. In general, most of the complete seizures were induced by less than 60 seconds of ILS, and they usually lasted less than 60 seconds.

The most effective frequency of ILS for inducing seizures in epileptic chickens at most ages was found to range from 10 to 20 fps. However, at one day and at or after 52 weeks of age birds were also highly sensitive to ILS frequencies above or below the optimum range.

The seizure latency was also affected by the flash frequency of ILS. The incidence of seizures with a short latency (20 seconds or less) was higher when high ILS frequencies were used, and lower when low ILS frequencies were used. The incidence of seizures with a long latency (more than 20 seconds) was higher when low ILS frequencies were used and lower when high ILS frequencies were used. Seizure duration was not affected by frequency of ILS except in birds tested at one day and four, six and seven weeks of age.

No obvious differences were found in seizure susceptibility, incidence of complete and incomplete seizures, and seizure latency between male and female chickens, but males tended to have longer seizures than females.

No prominent effect of parental genotype was found on the response of epileptic chickens to ILS.

Prolonged ILS after the initiation of a seizure had no effect on the duration of that seizure and the post-seizure depression, but the incidence of post-seizure depression was significantly increased in birds subjected to prolonged ILS. The post-seizure depression usually lasted less than ten minutes in both prolonged stimulation and control groups.

Prior exposure of epileptic chickens to ineffective ILS (2 fps) and to heat stress, did not affect seizure susceptibility of the birds exposed to effective ILS (14 fps) which followed immediately. Seizure susceptibility diminished significantly in day-old epileptic chicks which had been previously subjected to cold stress, and in birds at one day and older ages which had been previously subjected to emotional disturbances.

The time interval needed for day-old epileptic chicks to respond to a second ILS was much shorter than in birds at other ages. Almost all of the day-old chicks responded to a second ILS if given five minutes of rest

after the first seizure, and even 40 to 60 per cent of them responded to a second ILS immediately after the first seizure. In birds tested at four and eight weeks of age, 15 or 30 minutes of rest after the first seizure was usually long enough for most of the birds to respond to a second ILS. As many as eight successive seizures could be induced from a bird if effective ILS frequencies were used and the resting period after a seizure was long enough.

In Part III, segregation of the epi gene and its effects on sex distribution, fertility, embryonic mortality and hatchability were studied. The present data agree with those of Crawford (1969, 1970) indicating that a single autosomal recessive gene is responsible for epileptiform seizures in chickens. The expressivity of the gene was complete if an effective inducing method such as intermittent light stimulation was used.

The sex distribution in epileptic and in carrier chickens obtained from various mutant matings agreed with the expected 1 : 1 ratio.

Presence of the epi gene had no effect on fertility, embryonic mortality and hatchability.

In Part IV, electroencephalograms (EEG) of epileptic, carrier and normal chickens were studied and compared. The resting EEG of epileptic chickens was characterized by waves of relatively low frequency and high amplitude compared to the EEG of carrier and normal chickens.

Abnormal spiking patterns prior to the onset of severe clonic convulsive movements were found in the EEG of epileptic chickens exposed to ILS. The spiking frequency was identical to the flash frequency of ILS. No abnormal spiking was found in the EEG of carrier and normal chickens.

The wave frequency and amplitude in the resting EEG and in the EEG during ILS did not differ between carrier and normal chickens, except that the resting EEG recorded from leads A and B and the EEG during ILS recorded from lead A had faster wave frequency in normal chickens than in carriers.

No significant differences were found between the resting EEG and the EEG during ILS in normal and in carrier chickens.

The epileptiform seizures observed in chickens were found to be similar in many ways to grand mal seizures in humans. Both human epileptics and epileptic chickens are highly sensitive to ILS, and the optimum frequency range of ILS for human and epileptic chickens is similar. Abnormal spiking occurs in both humans and epileptic chickens during ILS. It seems that epileptic chickens may potentially be one of the best animal models for studying human epilepsy.

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Table 1: Numbers of birds used in each experiment.

Hatch no.	Hatching date	Total no. of birds	Expt.	Number of birds used in Part I 1	Part I 2	Part I 3	Part I 4	Part II 1	Part II 2	Part II 3	Part II 4	Part II 5	Part II 6	Part III	Part IV
P_1															
1	June 24/69	22			18			4							
2	July 22/69	13				9		4							
3	Sept. 9/69	36		36											
4	Sept. 27/69	41		41											
P_2															
5	Feb. 14/70	16						10							6
6	Mar. 1/70	11		10											1
7	Mar. 17/70	33				33									
8	Apr. 21/70	32						25							7
9	May 11/70	21						21							
10	May 18/70	20						20							
11	May 25/70	19						19	5	7					
12	June 1/70	28						21	4	9					
13	June 15/70	14						14		8					
14	June 22/70	36						36	10	19	8				
15	June 29/70	25						25							
16	July 6/70	33						33		13					
17	July 14/70	26						26			13				
18	July 31/70	27						27		4	13				
19	Aug. 16/70	20						20							
20	Sept. 3/70	42					42	42	23	30	7				7
21	Sept. 8/70	33						33	26	31					
22	Sept. 12/70	46						46	25	38					
23	Sept. 22/70	20						20							
24	Oct. 26/70	27						27							
25	Nov. 9/70	26						26							

(continued...)

Table 1: Numbers of birds used in each experiment (continued)

Hatch no.	Hatching date	Total no. of birds	Expt.	Number of birds used in Part I 1	Part I 2	Part I 3	Part I 4	Part II 1	Part II 2	Part II 3	Part II 4	Part II 5	Part II 6	Part III	Part IV
$P_2 + P_3$															
26	Mar. 2/71	54		29				35				40	25		
27	Mar. 17/71	44		14				7				14	30		
28	Mar. 20/71	25											25		
29	Mar. 30/71	36										36			
30	Apr. 13/71	69		30								69			
31	Apr. 27/71	28				28	28								
32	May 5/71	18										18			
33	May 18/71	52				52	52								
34	June 1/71	34				34	34								
35	June 6/71	35				35	35								
36	June 15/71	48				48	48								
37	Sept. 28/71	95												95	
38	Oct. 5/71	104												104	
39	Oct. 12/71	109												109	
40	Oct. 19/71	74												74	
41	Oct. 26/71	62												62	
42	Nov. 1/71	50												50	
43	Nov. 9/71	66												66	
44	Nov. 16/71	70												70	
45	Nov. 23/71	76												76	
46	Nov. 30/71	69												69	
47	Dec. 7/71	68												68	
48	Dec. 14/71	72												72	
Total		2025		160	18	239	239	541	93	159	41	177	80	915	21

Table 2: Response of chickens to heat and cold stimulation at different ages.

Stimulation	Maximum time of stimulation (mins.)	Phenotype	Birds tested (no.)	Age (days)	Birds responding (no.)	Birds responding (%)	Probability
Heat	3	Epileptic	31	1	27	87.1	< 0.01*
			5	3	3	60.0	
			5	5	5	100.0	
			14	7	10	71.4	
			7	14	2	28.6	
			14	28	4	28.6	
		Normal	20	1	0	0.0	--
			5	7	0	0.0	
Cold	10	Epileptic	25	1	0	0.0	--
			27	30	0	0.0	
		Normal	5	1	0	0.0	--
			2	30	0	0.0	

*χ^2 = 22.40; df = 5.

Table 3: Response of chickens to olfactory stimulation.

Type of stimulation	Maximum time of stimulation (mins.)	Age (days)	Epileptic		Normal	
			Birds tested (no.)	Birds responding (no.)	Birds tested (no.)	Birds responding (no.)
Dimethyl-sulfide	20	1	16	0	2	0
		3	16	0	2	0
		5	16	0	2	0
		7	16	0	2	0
Indian incense	20	1	16	0	2	0
		3	16	0	2	0
		5	16	0	2	0
		7	16	0	2	0

Table 4: Response of chickens to auditory stimulation.

Type of stimulation	Maximum time of stimulation (mins.)	Age (days)	Epileptic			Normal		
			Birds tested (no.)	Birds responding (no.)	(%)	Birds tested (no.)	Birds responding (no.)	(%)
Banging a wire screen on top of chick battery*	5	1	38	23	60.5	4	0	0.0
		3	38	18	47.4	4	0	0.0
		5	33	7	21.2	4	0	0.0
		7	33	2	6.1	4	0	0.0
		14	33	13	39.4	4	0	0.0
		21	31	19	61.3	4	0	0.0
		28	31	20	64.5	4	0	0.0
Pure-tone from audiogenerator	5	1	38	0	0.0	4	0	0.0
		3	38	0	0.0	4	0	0.0
		5	33	0	0.0	4	0	0.0
		7	33	0	0.0	4	0	0.0
		14	33	0	0.0	4	0	0.0
		21	31	0	0.0	4	0	0.0
		28	31	0	0.0	4	0	0.0

*X^2 = 40.07; df = 6; $P < 0.01$ for epileptic chickens responding at different ages.

Table 5: Response of day-old epileptic chicks to two different methods of complex sound stimulation.

Hatch no.	Complex sound stimulation produced by banging a wire screen on top of the chick battery			Complex sound stimulation produced by banging a wire screen beside the chick battery			χ^2	df	Probability
	Birds tested (no.)	Birds responding (no.)	(%)	Birds tested (no.)	Birds responding (no.)	(%)			
31	28	10	35.7	28	0	0.0	12.17	1	<0.01
33	52	30	57.7	52	0	0.0	42.16	1	<0.01
34	34	13	38.2	34	2	5.9	10.34	1	<0.01
35	35	13	37.1	35	6	17.1	3.54	1	<0.10
36	48	23	47.9	48	3	6.3	19.78	1	<0.01
Total	197	89	45.2	197	11	5.6	81.54	1	<0.01

Table 6: Response of day-old epileptic chicks to dark and bright environments and to intermittent light stimulation (14 fps).

Hatch no.	From incubator to dark room: Five minutes in the dark — Birds tested (no.)	Birds responding (no.)	Birds responding (%)	From incubator to dark room: Light turned on after 5 minutes — Birds tested (no.)	Birds responding (no.)	Birds responding (%)	From incubator to bright room — Birds tested (no.)	Birds responding (no.)	Birds responding (%)	Intermittent light stimulation (14 fps) — Birds tested (no.)	Birds responding (no.)	Birds responding (%)
31	28	0	0.0	28	15	53.6	-	-	-	28	27	96.4
33	52	0	0.0	52	31	59.6	-	-	-	52	49	94.2
34	34	1	2.9	33	20	60.6	-	-	-	34	34	100.0
35	-	-	-	-	-	-	35	26	74.3	35	27	77.1
36	-	-	-	-	-	-	48	40	83.3	48	47	97.9
Total	114	1	0.9	113	66	58.4	83	66	79.5	197	184	93.4

Table 7: Chi-square test of independence of the comparison between responses of day-old epileptic chicks to dark and bright environments and intermittent light stimulation.

Comparison between observations		Hatch no. 31	Hatch no. 33	Hatch no. 34	Hatch no. 35	Hatch no. 36	Total
(1) vs. (4)	χ^2	52.14	92.65	29.24	-	-	256.51
	P	<0.01	<0.01	<0.01	-	-	<0.01
(2) vs. (4)	χ^2	13.71	17.55	16.62	-	-	56.34
	P	<0.01	<0.01	<0.01	-	-	<0.01
(3) vs. (4)	χ^2	-	-	-	0.08	5.59	11.77
	P	-	-	-	<0.90	<0.05	<0.01
(1) vs. (2)	χ^2	-	-	-	-	-	90.28
	P	-	-	-	-	-	<0.01
(1) vs. (3)	χ^2	-	-	-	-	-	132.34
	P	-	-	-	-	-	<0.01
(2) vs. (3)	χ^2	-	-	-	-	-	9.90
	P	-	-	-	-	-	<0.01

df = 1 for all comparisons.

(1) Response of day-old epileptic chicks which were removed from the darkened or dimly lighted hatching compartment of the incubator to a dark environment.

(2) Response of day-old epileptic chicks which remained in the dark for five minutes after removal from the incubator and then were exposed to five minutes of bright light.

(3) Response of day-old epileptic chicks which were removed from the darkened or dimly lighted hatching compartment of the incubator to a bright environment.

(4) Response of day-old epileptic chicks to intermittent light stimulation of 14 fps.

Table 8: Response of chickens to intermittent light stimulation (14 fps).

Phenotype	Maximum time of stimulation (mins.)	Birds tested (no.)	Age (days)	Birds responding (no.)	Birds responding (%)	χ^2	df	Probability
Epileptic	3	30	1	30	100.0	72.09	6	< 0.01
		30	3	11	36.7			
		28	5	13	46.4			
		25	7	12	48.0			
		24	14	22	91.7			
		24	21	24	100.0			
		24	28	24	100.0			
Normal	3	12	1	0	0.0	-	-	-
		12	3	0	0.0			
		12	5	0	0.0			
		12	7	0	0.0			
		12	14	0	0.0			
		12	21	0	0.0			
		12	28	0	0.0			

Table 9: Comparison of epileptic chickens responding to heat, auditory and intermittent light stimulation.

Age of the birds tested (days)	Percentage of birds responding to			χ^2	df	Probability
	Heat stimulation	Auditory stimulation	Intermittent light stimulation			
1	87.1	60.5	100.0	18.01	2	<0.01
3	60.0	47.4	36.7	1.43	2	<0.50
5	100.0	21.2	46.4	12.94	2	<0.01
7	71.0	6.1	48.0	22.48	2	<0.01
14	28.6	39.4	91.7	18.37	2	<0.01
21	--	61.3	100.0	11.85	1	<0.01
28	28.6	64.5	100.0	21.87	2	<0.01

Table 10: The incidence of complete and incomplete seizures of epileptic chickens in response to intermittent light stimulation (ILS) at various flash frequencies and at different ages.

Age	Flash frequency of ILS														
	2 fps			5 fps			8 fps			10 fps			12 fps		
	Birds tested (no.)	CS (%)	IS (%)	Birds tested (no.)	CS (%)	IS (%)	Birds tested (no.)	CS (%)	IS (%)	Birds tested (no.)	CS (%)	IS (%)	Birds tested (no.)	CS (%)	IS (%)
1 day	40	97.5	0.0	122	94.3	1.6	46	95.7	4.3	22	100.0	0.0	52	94.2	0.0
3 days	34	0.0	8.8	111	28.8	11.7	50	22.0	24.0	25	28.0	8.0	55	7.3	20.0
5 days	32	6.3	12.5	113	10.6	9.7	45	33.0	17.8	19	15.8	10.5	50	12.0	10.0
7 days	34	0.0	0.0	112	10.7	11.6	42	57.1	21.4	23	34.8	8.7	50	16.0	8.0
2 wks.	34	0.0	0.0	100	46.0	16.0	35	42.9	11.4	13	69.2	7.7	46	71.7	10.9
3 wks.	30	0.0	0.0	103	38.8	9.7	40	77.5	15.0	25	76.0	12.0	48	91.7	2.1
4 wks.	90	2.2	6.7	104	58.7	12.5	18	66.7	11.1	18	66.7	33.3	43	88.4	7.0
5 wks.	31	0.0	0.0	103	62.5	5.8	38	78.9	10.5	22	77.3	18.2	43	83.7	9.3
6 wks.	31	0.0	0.0	102	62.8	8.8	35	74.3	17.1	22	90.9	9.1	43	83.7	4.7
7 wks.	31	0.0	0.0	102	71.6	4.9	36	63.9	19.4	21	85.7	14.3	41	97.6	2.4
8 wks.	87	0.0	2.3	98	69.4	14.3	35	77.1	11.4	21	95.2	4.8	38	92.1	7.9
26 wks.	9	0.0	0.0	55	72.8	12.7	9	66.7	11.1	6	83.3	16.7	9	77.8	22.2
52 wks.	20	0.0	0.0	20	90.0	0.0	20	95.0	5.0	20	100.0	0.0	20	100.0	0.0
104 wks.	8	0.0	0.0	8	100.0	0.0	8	100.0	0.0	8	100.0	0.0	8	100.0	0.0

(continued . . .)

(fps = flashes per second, CS = complete seizure, IS = incomplete seizure)

Table 10: continued.

Age	Flash frequency of ILS											
	14 fps			20 fps			30 fps			40 fps		
	Birds tested (no.)	CS (%)	IS (%)	Birds tested (no.)	CS (%)	IS (%)	Birds tested (no.)	CS (%)	IS (%)	Birds tested (no.)	CS (%)	IS (%)
1 day	65	98.5	1.5	65	98.5	1.5	33	72.7	12.1	11	52.4	0.0
3 days	62	22.6	8.1	55	40.0	10.9	-	-	-	-	-	-
5 days	50	42.0	6.0	55	10.9	16.4	-	-	-	-	-	-
7 days	59	17.0	17.0	56	16.1	10.7	-	-	-	-	-	-
2 wks.	50	76.0	16.0	48	60.4	22.9	-	-	-	-	-	-
3 wks.	57	84.2	8.8	52	71.2	15.4	-	-	-	-	-	-
4 wks.	56	92.9	1.8	52	88.5	7.7	57	66.7	17.5	60	38.3	15.0
5 wks.	56	76.8	12.5	52	75.0	19.2	-	-	-	-	-	-
6 wks.	55	90.9	0.0	50	86.0	10.0	-	-	-	-	-	-
7 wks.	55	94.6	3.6	50	94.0	4.0	-	-	-	-	-	-
8 wks.	51	96.1	2.0	50	90.0	6.0	56	80.4	8.9	56	53.6	14.3
26 wks.	55	100.0	0.0	55	98.2	0.0	14	85.7	7.1	14	85.7	7.1
52 wks.	20	100.0	0.0	20	100.0	0.0	20	100.0	0.0	20	100.0	0.0
104 wks.	8	100.0	0.0	8	100.0	0.0	8	100.0	0.0	8	100.0	0.0

(fps = flashes per second, CS = complete seizure, IS = incomplete seizure)

Table 11: Chi-square test of homogeneity of the response of epileptic chickens at different ages to intermittent light stimulation.

Ages of birds tested	ILS frequency fps	Seizure susceptibility			Complete seizures			Incomplete seizures		
		χ^2	df	P	χ^2	df	P	χ^2	df	P
A	5	307.45	13	<0.01	341.02	13	<0.01	26.52	13	<0.05
A	8	95.73	13	<0.01	107.70	13	<0.01	15.42	13	<0.50
A	10	128.40	13	<0.01	99.76	13	<0.01	17.71	13	<0.25
A	12	300.19	13	<0.01	307.42	13	<0.01	27.42	13	<0.05
A	14	300.84	13	<0.01	315.84	13	<0.01	40.33	13	<0.01
A	20	302.86	13	<0.01	292.89	13	<0.01	37.02	13	<0.01
B	30	7.29	5	<0.25	13.55	5	<0.05	7.00	5	<0.25
B	40	25.38	5	<0.01	35.03	5	<0.01	8.30	5	<0.25

(fps = flashes per second, ILS = intermittent light stimulation)

A: Birds were tested from one day to seven days of age at two-day intervals, and from two to eight weeks of age at weekly intervals, and then at 26,52 and 104 weeks of age.

B: Birds were tested at one day and at four, eight, 26, 52 and 104 weeks of age.

Table 12: Chi-square test of homogeneity of the response of epileptic chickens to different intermittent light stimulation frequencies at various ages.

ILS frequencies of birds tested	Age of birds tested	Seizure susceptibility			Complete seizures			Incomplete seizures		
		χ^2	df	P	χ^2	df	P	χ^2	df	P
A	1 day	92.42	8	<0.01	81.82	8	<0.01	18.73	8	<0.05
B	3 days	9.68	5	<0.10	17.44	5	<0.01	9.38	5	<0.10
B	5 days	24.02	5	<0.01	32.45	5	<0.01	5.20	5	<0.50
B	7 days	48.32	5	<0.01	45.32	5	<0.01	4.90	5	<0.50
B	2 wks.	26.98	5	<0.01	20.33	5	<0.01	3.90	5	<0.75
B	3 wks.	73.72	5	<0.01	61.58	5	<0.01	6.12	5	<0.50
C	4 wks.	61.47	7	<0.01	64.10	7	<0.01	17.47	7	<0.05
B	5 wks.	24.94	5	<0.01	7.56	5	<0.25	7.51	5	<0.25
B	6 wks.	26.61	5	<0.01	23.67	5	<0.01	10.17	5	<0.10
B	7 wks.	33.33	5	<0.01	34.13	5	<0.01	14.56	5	<0.05
C	8 wks.	30.22	7	<0.01	40.02	7	<0.01	9.16	7	<0.25
C	26 wks.	18.33	7	<0.05	30.27	7	<0.01	17.38	7	<0.05
C	52 wks.	19.23	7	<0.01	12.26	7	<0.10	7.30	7	<0.50
-	104 wks.	-	-	-	-	-	-	-	-	-

A: Birds were tested with ILS (intermittent light stimulation) frequencies of 2, 5, 8, 10, 12, 14, 20, 30 and 40 fps (flashes per second).
B: Birds were tested with ILS of 5, 8, 10, 12, 14 and 20 fps.
C: Birds were tested with ILS of 5, 8, 10, 12, 14, 20, 30 and 40 fps.

Table 13: Distribution of latencies of light-induced seizures in epileptic chickens at different ages.

Age	Total no. of seizures	Percentage of seizures with latency of: 1 - 10 sec.	11 - 20 sec.	21 - 30 sec.	31 - 40 sec.	41 - 60 sec.	61 - 120 sec.
1 day	358	3.6	34.6	44.1	11.2	6.5	0.0
3 days	90	3.3	19.0	33.3	25.6	14.4	4.4
5 days	63	0.0	20.6	31.7	28.6	15.9	3.2
7 days	71	1.4	15.5	43.6	25.4	12.7	1.4
2 wks.	170	0.0	28.2	41.2	22.4	7.7	0.5
3 wks.	219	0.4	28.3	54.8	11.4	5.1	0.0
4 wks.	221	0.9	29.0	49.8	15.4	4.5	0.4
5 wks.	230	0.9	43.0	31.8	17.8	3.9	2.6
6 wks.	239	3.8	45.6	36.4	10.9	3.3	0.0
7 wks.	253	1.2	49.0	30.8	15.0	4.0	0.0
8 wks.	244	1.2	51.2	32.0	10.7	4.6	0.3
26 wks.	167	9.6	62.9	23.4	3.6	0.5	0.0
52 wks.	117	17.9	68.4	11.1	2.6	0.0	0.0
104 wks.	48	54.2	45.8	0.0	0.0	0.0	0.0
Total	2490	4.0	40.3	36.4	13.5	5.2	0.6

Table 14: Distribution of latencies of light-induced seizures of epileptic chickens subjected to different frequencies of intermittent light stimulation.

Stim. freq. (fps)	Total no. of seizures	Percentage of seizures with latency of:					
		1 - 10 sec.	11 - 20 sec.	21 - 30 sec.	31 - 40 sec.	41 - 60 sec.	61 - 120 sec.
5	654	0.8	17.0	47.2	22.8	10.8	1.4
8	291	3.1	27.8	45.4	19.9	3.8	0.0
10	188	3.2	35.6	39.4	17.0	3.7	1.1
12	364	2.7	42.3	40.1	10.2	4.4	0.3
14	524	5.7	55.9	27.9	7.3	2.9	0.3
20	469	8.6	63.3	21.3	4.7	1.7	0.4
Total	2490	4.0	40.3	36.4	13.5	5.2	0.6

Table 15: Average seizure latency (in seconds) of epileptic chickens subjected to various flash frequencies of intermittent light stimulation at different ages.

Age	Frequency of intermittent light stimulation									
	5 fps		8 fps		10 fps		12 fps		14 fps	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1 day	26.8	1.0	25.9	1.0	21.8	1.7	25.9	1.3	20.5	0.8
3 days	35.1	1.7	28.9	1.5	27.0	3.4	25.3	2.8	27.6	3.6
5 days	47.3	4.7	32.2	2.9	30.0	2.5	35.5	4.9	24.8	1.9
7 days	34.3	2.4	29.6	1.6	27.8	4.2	36.0	8.2	28.2	1.1
2 wks.	33.0	2.2	29.1	2.2	33.0	1.8	27.1	1.4	23.7	1.6
3 wks.	30.4	1.3	24.8	1.2	24.4	1.8	25.8	1.2	22.7	0.3
4 wks.	30.7	1.1	32.1	3.1	26.5	1.4	25.1	1.1	22.9	0.9
5 wks.	32.7	1.6	22.9	1.0	27.9	1.7	23.0	1.7	20.5	1.8
6 wks.	28.5	0.9	24.4	0.8	22.7	1.7	20.5	1.4	17.1	1.0
7 wks.	28.8	0.9	24.0	1.1	26.9	2.3	18.9	1.0	19.7	1.3
8 wks.	27.5	0.8	26.4	1.5	27.6	2.2	19.0	2.8	19.6	1.6
26 wks.	23.7	1.3	17.8	1.8	17.4	2.5	14.1	2.2	15.4	0.7
52 wks.	20.6	1.4	16.9	1.7	14.9	0.8	14.8	0.9	11.9	0.5
104 wks.	17.3	0.4	11.1	0.9	11.1	0.7	10.8	0.4	8.5	0.3
Average latency	29.3	0.4	25.2	0.5	23.9	0.7	22.9	0.6	20.2	0.2
Total no. of seizures measured	654		291		188		364		524	

(The average seizure latency of 39 birds responding to intermittent light stimulation of 2 fps at one day of age was 30.9 $\pm$ 2.0 seconds.)

(continued . . .)

Table 15: continued.

Age	Frequency of intermittent light stimulation						Total seizures measured (no.)	Seizure latency	
	20 fps		30 fps		40 fps				
	Mean	SE	Mean	SE	Mean	SE		Mean	SE
1 day	20.8	1.0	19.4	1.2	25.6	3.6	432*	24.5	0.5
3 days	29.6	3.6	-		-		90	31.0	1.3
5 days	26.5	3.7	-		-		63	32.3	1.7
7 days	25.0	3.5	-		-		71	30.1	1.4
2 wks.	19.9	0.8	-		-		170	27.2	0.9
3 wks.	20.2	0.8	-		-		219	24.8	0.5
4 wks.	17.8	0.8	19.6	0.7	23.3	1.7	282	24.3	0.5
5 wks.	17.7	1.0	-		-		230	24.7	0.8
6 wks.	17.9	0.9	-		-		239	22.1	0.5
7 wks.	16.5	0.7	-		-		253	22.5	0.6
8 wks.	17.7	2.5	16.2	0.5	15.7	1.2	319	21.2	0.6
26 wks.	16.2	0.8	13.3	0.9	12.7	1.0	191	17.2	0.5
52 wks.	11.2	0.8	9.8	0.5	10.9	0.5	157	13.7	0.4
104 wks.	8.3	0.6	7.8	0.4	8.0	0.6	64	10.3	0.4
Seizure latency	18.6	0.6	16.0	0.5	16.6	0.8	-	23.0	0.2
Total seizures measured (no.)	469		147		104		2780*		

* Data of birds subjected to intermittent light stimulation of 2 fps were included.

Table 16: Analyses of variance of effect of age on seizure latency and seizure duration of epileptic chickens subjected to various intermittent light stimulation frequencies.

Ages of birds tested	ILS frequency fps		df	Seizure latency		Seizure duration	
				Mean square	F	Mean square	F
A	5	Age	13	892.71	9.39**	197143.87	9.07**
		Error	640	95.08		16216.81	
A	8	Age	13	438.70	8.88**	18526.79	2.52**
		Error	277	49.38		7355.63	
A	10	Age	13	393.20	6.80**	165864.94	3.39**
		Error	174	57.87		48934.58	
A	12	Age	13	640.30	6.95**	8223.72	1.51
		Error	350	92.13		5445.83	
A	14	Age	13	579.74	8.74**	31287.05	3.15**
		Error	510	66.31		9929.21	
A	20	Age	13	487.88	2.98**	44842.61	6.48**
		Error	455	163.59		6915.45	
B	30	Age	5	438.62	27.13**	24398.95	3.71**
		Error	141	16.17		6570.09	
B	40	Age	5	673.98	17.92**	3527.25	1.63
		Error	98	37.61		2170.10	

** $P < 0.01$

A: Birds were tested from one day to seven days of age at two-day intervals, and from two to eight weeks of age at weekly intervals, and then at 26, 52 and 104 weeks of age.

B: Birds were tested at one day and at four, eight, 26, 52 and 104 weeks of age.

Table 17: Analyses of variance of effect of frequency of intermittent light stimulation (ILS) on seizure latency and seizure duration of epileptic chickens tested at various ages.

ILS frequencies of birds tested	Age		df	Seizure latency		Seizure duration	
				Mean square	F	Mean square	F
A	1 day	Frequency	8	633.68	7.52**	88427.95	2.37*
		Error	423	84.31		37298.32	
B	3 days	Frequency	5	183.80	1.32	445.80	1.04
		Error	84	139.69		428.88	
B	5 days	Frequency	5	830.90	6.56**	135.37	0.51
		Error	57	126.69		264.59	
B	7 days	Frequency	5	162.85	1.24	337.78	0.97
		Error	65	131.47		348.88	
B	2 wks.	Frequency	5	788.98	7.45**	8340.10	1.07
		Error	164	105.84		7770.15	
B	3 wks.	Frequency	5	438.76	9.32**	968.00	1.63
		Error	213	47.07		594.83	
C	4 wks.	Frequency	7	882.36	18.03**	1402.62	1.74*
		Error	274	48.93		804.93	

* $P<0.05$; ** $P<0.01$. (continued . . .)

A: Birds were tested with ILS frequencies of 2, 5, 8, 10, 12, 14, 20, 30, and 40 fps (flashes per second). B: Birds were tested with ILS of 5, 8, 10, 12, 14 and 20 fps. C: Birds were tested with ILS of 5, 8, 10, 12, 14, 20, 30 and 40 fps.

Table 17: continued.

ILS frequencies of birds tested	Age		df	Seizure latency		Seizure duration	
				Mean square	F	Mean square	F
B	5 wks.	Frequency	5	1441.01	14.27**	4746.06	0.39
		Error	224	100.98		12252.89	
B	6 wks.	Frequency	5	960.88	19.97**	24383.89	2.37*
		Error	233	48.12		10269.46	
B	7 wks.	Frequency	5	1168.55	21.46**	73062.05	3.09**
		Error	247	54.45		23643.84	
C	8 wks.	Frequency	7	1012.77	9.14**	4601.92	1.69
		Error	311	110.86		2725.49	
C	26 wks.	Frequency	7	340.90	9.89**	3849.93	0.48
		Error	183	34.48		8010.42	
C	52 wks.	Frequency	7	248.73	14.26**	18528.58	1.40
		Error	149	17.44		13229.64	
C	104 wks.	Frequency	7	66.81	16.06**	916.14	1.25
		Error	56	4.16		735.80	

* $P<0.05$; ** $P<0.01$.
A: Birds were tested with ILS frequencies of 2, 5, 8, 10, 12, 14, 20, 30 and 40 fps (flashes per second). B: Birds were tested with ILS of 5, 8, 10, 12, 14 and 20 fps. C: Birds were tested with ILS of 5, 8, 10, 12, 14, 20, 30 and 40 fps.

Table 18: Distribution of durations of light-induced seizures of epileptic chickens at different ages.

Age	Total no. of seizures	Percentage of seizures with duration of:			
		1 - 60 sec.	61 - 120 sec.	121 - 600 sec.	over 600 sec.
1 day	358	32.1	37.4	24.6	5.9
3 days	90	77.8	22.2	0.0	0.0
5 days	63	87.3	12.7	0.0	0.0
1 wk.	71	73.2	26.8	0.0	0.0
2 wks.	170	64.1	35.3	0.0	0.6
3 wks.	219	58.9	40.2	0.9	0.0
4 wks.	221	67.4	32.1	0.5	0.0
5 wks.	230	67.8	30.0	1.7	0.5
6 wks.	239	70.3	27.2	2.1	0.4
7 wks.	253	69.2	26.5	3.6	0.7
8 wks.	244	75.8	20.9	2.9	0.4
26 wks.	167	86.8	8.4	4.2	0.6
52 wks.	117	94.9	0.0	4.3	0.8
104 wks.	48	97.9	0.0	2.1	0.0
Total	2490	66.9	26.8	5.2	1.1

Table 19: Distribution of duration of light-induced seizures of epileptic chickens subjected to different frequencies of intermittent light stimulation.

Stim. freq. (fps)	Total no. of seizures	Percentage of seizures with duration of:			
		1 - 60 sec.	61 - 120 sec.	121 - 600 sec.	over 600 sec.
5	654	70.6	22.0	5.2	2.2
8	291	56.7	38.1	4.5	0.7
10	188	61.7	30.9	5.3	2.1
12	364	57.6	36.3	6.0	0.6
14	524	71.4	23.3	4.4	0.9
20	469	72.7	21.1	5.6	0.6
Total	2490	66.9	26.8	5.2	1.1

Table 20: Average seizure duration (in seconds) of epileptic chickens subjected to various flash frequencies of intermittent light stimulation at different ages.

Age	Frequency of intermittent light stimulation									
	5 fps		8 fps		10 fps		12 fps		14 fps	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1 day	194.7	24.4	130.3	27.8	173.3	54.8	89.5	11.3	131.5	16.7
3 days	55.5	3.3	46.6	6.0	40.7	5.8	44.5	11.3	44.7	6.2
5 days	47.9	4.9	42.3	3.7	35.5	4.9	47.5	11.0	39.6	2.7
1 wk.	45.3	4.2	59.9	4.5	54.6	4.6	52.0	5.3	42.4	7.3
2 wks.	59.5	2.7	66.4	5.8	54.3	2.6	95.1	34.3	53.5	2.1
3 wks.	57.8	2.6	70.7	4.2	62.3	4.3	62.9	13.8	55.8	1.6
4 wks.	53.0	2.0	65.4	7.0	49.3	3.7	66.6	4.0	56.3	1.4
5 wks.	69.9	22.1	65.8	3.8	59.9	3.5	71.7	8.9	83.1	18.0
6 wks.	56.3	9.8	80.7	14.4	134.0	69.0	64.5	3.7	54.0	5.0
7 wks.	48.3	1.4	60.2	6.1	204.9	130.5	67.7	3.6	72.3	11.6
8 wks.	48.0	1.2	76.8	21.9	73.4	9.3	73.4	6.3	53.2	2.2
26 wks.	45.3	4.6	60.7	30.9	58.0	29.9	88.4	57.2	62.7	18.4
52 wks.	52.4	12.1	28.4	1.6	28.8	1.8	28.6	1.7	116.5	71.1
104 wks.	25.8	2.7	23.1	1.6	53.9	26.5	22.0	1.7	22.4	2.1
Seizure duration	78.7	5.4	71.5	5.2	90.9	16.1	68.6	3.9	69.7	4.5
Total seizures measured (no.)	654		291		188		364		524	

(The average seizure duration of 39 birds responding to intermittent light stimulation of 2 fps at one day of age was 86.1 $\pm$ 16.7 seconds.)

(continued . . .)

Table 20: continued.

Age	Frequency of intermittent light stimulation						Total seizures measured (no.)	Seizure duration	
	20 fps		30 fps		40 fps				
	Mean	SE	Mean	SE	Mean	SE		Mean	SE
1 day	153.0	24.8	120.8	31.9	58.3	8.8	432*	142.2	9.4
3 days	50.7	4.9	-		-		90	49.9	2.2
5 days	44.3	5.8	-		-		63	43.2	2.0
7 days	47.2	4.7	-		-		71	50.2	2.2
2 wks.	52.3	2.1	-		-		170	64.3	4.6
3 wks.	60.6	7.4	-		-		219	61.1	1.7
4 wks.	58.3	1.8	50.7	1.5	46.5	3.6	282	55.8	1.0
5 wks.	50.5	2.7	-		-		230	68.0	7.3
6 wks.	50.3	3.9	-		-		239	65.1	6.7
7 wks.	65.1	10.2	-		-		253	71.6	9.9
8 wks.	62.9	12.0	55.5	8.0	53.0	2.0	319	59.2	3.0
26 wks.	47.5	4.0	33.0	2.3	69.0	38.1	191	54.1	6.4
52 wks.	30.6	2.1	34.4	1.7	30.1	2.4	157	43.7	9.4
104 wks.	23.5	2.0	31.4	2.1	25.6	2.4	64	28.5	3.4
Seizure duration	67.0	4.1	58.9	7.0	47.5	4.6		71.7	2.2
Total seizures measured (no.)	469		147		104		2780*		

* Data for birds subjected to intermittent light stimulation of 2 fps were included.

Table 21: Sex effect on response of epileptic chickens to intermittent light stimulation.

ILS frequency, fps		Male	Female	χ^2	df	Probability
5	Trials (no.)	563	633			
	Susceptibility (%)	64.3	58.9	3.66	1	<0.10
	Complete seizure (%)	53.1	49.9	1.22	1	<0.50
	Incomplete seizure (%)	11.2	9.0	1.60	1	<0.25
14	Trials (no.)	235	355			
	Susceptibility (%)	89.4	89.6	0.01	1	<0.95
	Complete seizure (%)	85.1	83.4	0.31	1	<0.75
	Incomplete seizure (%)	4.3	6.2	1.04	1	<0.50
20	Trials (no.)	339	292			
	Susceptibility (%)	81.7	80.1	0.25	1	<0.75
	Complete seizure (%)	70.5	70.9	0.01	1	<0.95
	Incomplete seizure (%)	11.2	9.2	0.21	1	<0.75

Table 22: Average seizure latency and average seizure duration of male and female epileptic chickens.

ILS frequency (fps)	Sex	Number of seizures	Average latency (sec.)	Standard error	Average duration (sec.)	Standard error
5	Male	299	28.8	0.6	96.9	12.8
	Female	316	29.7	0.5	68.0	16.8
14	Male	200	17.7	0.5	65.3	8.9
	Female	296	18.8	0.6	55.5	3.0
20	Male	239	18.1	0.4	93.2	23.3
	Female	207	18.6	0.5	62.9	4.5

Table 23: Analyses of variance of effect of sex on seizure latency and seizure duration of epileptic chickens.

ILS frequency, fps	Source of variation	df	Seizure latency		Seizure duration	
			Mean square	F	Mean square	F
5	Sex	1	135.44	1.54	128582.42	4.53*
	Error	613	88.01		28415.77	
14	Sex	1	144.14	1.90	10259.28	1.29
	Error	494	75.89		7948.58	
20	Sex	1	34.04	0.68	101483.45	1.42
	Error	444	50.37		71472.34	

* $P < 0.05$

Table 24: Distribution of seizure durations of male and female epileptic chickens.

Total number of seizures	Sex	Seizure with duration of: 1 - 60 sec.		61 - 120 sec.		121 - 600 sec.		601 - 900 sec.		Over 900 sec.	
		no.	%	no.	%	no.	%	no.	%	no.	%
738	Male	515	69.8	165	22.4	45	6.1	3	0.4	10	1.3
819	Female	581	70.9	199	24.3	33	4.1	5	0.6	1	0.1
χ^2		0.25		0.82		3.49		0.32		8.41	
df		1		1		1		1		1	
Probability		<0.75		<0.50		<0.10		<0.75		<0.01	

Table 25: Effect of parentage on response of epileptic chickens to intermittent light stimulation from one day to 56 days of age.

ILS frequency, fps		Parental cross (Male x Female) E x E	E x C	C x E	C x C	χ^2	df	Probability
5	Birds tested (no.)	46	17	34	25			
	Trials (no.)	435	186	286	240			
	Susceptibility (%)	62.1	48.9	58.7	71.7	23.71	3	<0.01
	Complete seizure (%)	52.6	39.8	49.0	60.4	11.47	3	<0.01
	Incomplete seizure (%)	9.5	9.1	9.7	11.3	0.88	3	<0.90
14	Birds tested (no.)	29	8	17	15			
	Trials (no.)	282	88	112	135			
	Susceptibility (%)	76.6	75.0	80.4	83.7	3.69	3	<0.50
	Complete seizure (%)	68.1	68.2	75.0	77.8	5.38	3	<0.25
	Incomplete seizure (%)	8.5	6.8	5.4	5.9	1.64	3	<0.75
20	Birds tested (no.)	29	9	14	13			
	Trials (no.)	241	89	121	134			
	Susceptibility (%)	73.0	79.8	95.9	81.3	26.94	3	<0.01
	Complete seizure (%)	61.4	62.9	71.9	71.6	33.27	3	<0.01
	Incomplete seizure (%)	11.6	16.9	24.0	9.7	13.51	3	<0.01

E = Epileptic; C = Carrier.

Table 26: Effect of parentage on seizure latency and seizure duration of epileptic chickens subjected to intermittent light stimulation from one day to 56 days of age.

ILS frequency (fps)	Parental cross (male x female)	Birds tested (no.)	Complete seizures (no.)	Seizure latency (sec.)		Seizure duration (sec.)	
				Mean	SE	Mean	SE
5	E x E	46	228	31.0	0.7	105.8[a]	13.4
	E x C	17	74	30.7	1.3	75.6[ab]	11.7
	C x E	34	140	30.0	0.8	73.8[b]	5.3
	C x C	25	145	28.9	0.8	60.8[b]	5.0
14	E x E	29	192	22.8[a]	0.7	74.9	6.9
	E x C	8	60	20.6[ab]	0.6	57.6	3.5
	C x E	17	84	21.5[ab]	1.0	69.7	8.5
	C x C	15	105	19.4[b]	0.7	65.3	4.3
20	E x E	29	148	20.0	0.7	74.8	8.1
	E x C	9	56	17.5	0.9	56.8	4.9
	C x E	14	87	17.1	1.2	71.3	6.3
	C x C	13	96	17.7	1.1	81.6	12.8

E = Epileptic; C = Carrier.
Averages with different superscripts within each column differ significantly ($P < 0.05$).

Table 27: Analyses of variance of effect of parentage on seizure latency and seizure duration of epileptic chickens.

ILS frequency (fps)	Source of variation	df	Seizure latency		Seizure duration	
			Mean square	F	Mean square	F
5	Parentage	3	140.42	1.31	68558.44	3.62*
	Error	583	107.29		18945.80	
14	Parentage	3	283.99	3.69*	5319.43	0.94
	Error	437	76.88		5635.36	
20	Parentage	3	212.90	2.30	7481.79	0.88
	Error	383	92.69		8549.43	

* $P < 0.05$

Table 28: Effect of prolonged intermittent light stimulation on the duration of depression of epileptic chickens after a seizure.

		Control					Prolonged stimulation				
Age (days)	Birds tested (no.)	Recovered immediately after seizure (no.)	Depressed immediately after seizure (no.)	Duration of depression (mins.) <5	<10	>10	Recovered immediately after seizure (no.)	Depressed immediately after seizure (no.)	Duration of depression (mins.) <5	<10	>10
21 - 22	7	5	2	1	1	0	0	7	5	2	0
42 - 46	13	6	7	3	3	1	1	12	9	2	1
56 - 58	13	7	6	4	1	1	1	12	6	4	2
69 - 70	8	6	2	2	0	0	1	7	0	5	2
Total	41	24	17	10	5	2	3	38	20	13	5
%		58.5	41.5*	58.8	29.4	11.8	7.3	92.7*	52.6	34.2	13.2

* $P < 0.01$ prolonged stimulation group vs. control ($\chi^2 = 24.35$, df = 1).

Table 29: Response of epileptic chickens to intermittent light stimulation (14 fps) after subjecting them to some stressful conditions.

Type of stress	Period of stress (mins.)	Birds tested (no.)	Age (days)	Birds responding to stress treatment (no.)	% of birds responding to ILS (14 fps)		χ^2 (df=1)	Probability
					Treatment	Control		
Heat stress 65° - 75°C	3	10	1	8	100.0	100.0	-	-
		14	28	4	100.0	100.0	-	-
Cold stress - 8°C	10	38	1	0	34.2	89.5	24.59	<0.01
		20	34	0	75.0	80.0	0.14	<0.75
		20	54	0	75.0	80.0	0.14	<0.75
Ineffective ILS (2fps)	10	20	35	0	100.0	100.0	-	-
Emotional disturbance	1	39	1	30	0.0	100.0	-	-
		36	30	4	15.6	84.4	30.25	<0.01
		20	42	0	35.0	100.0	19.26	<0.01

Table 30: Response of epileptic chickens to intermittent light stimulation at different time intervals following a seizure.

ILS (fps)	Time intervals following seizure (mins.)	One day of age			28 - 30 days of age			56 days of age		
		Birds tested (no.)	Birds responding (no.)	(%)	Birds tested (no.)	Birds responding (no.)	(%)	Birds tested (no.)	Birds responding (no.)	(%)
5	0	5	3	60	10	1	10	5	0	0
	5	5	5	100	8	2	25	5	2	40
	15	5	5	100	9	4	44	5	2	40
	30	5	5	100	8	4	50	5	5	100
	60	5	5	100	7	4	57	5	5	100
14	0	5	2	40	11	0	0	5	0	0
	5	5	5	100	10	6	60	5	3	60
	15	5	5	100	11	11	100	5	5	100
	30	5	5	100	10	10	100	5	5	100
	60	5	5	100	11	10	91	5	5	100
20	0	6	3	50	10	0	0	5	0	0
	5	6	6	100	10	6	60	5	3	60
	15	6	6	100	10	8	80	5	2	40
	30	6	5	83	10	10	100	5	5	100
	60	6	6	100	9	9	100	5	5	100
Combined	0	16	8	50	31	1	3	15	0	0
	5	16	16	100	28	14	50	15	8	53
	15	16	16	100	30	23	67	15	9	60
	30	16	15	94	28	24	86	15	15	100
	60	16	16	100	27	23	85	15	15	100
χ^2				30.35			51.21			169.50
df				4			4			4
Probability				<0.01			<0.01			<0.01

Table 31: Average seizure latency and duration of two successive seizures induced by intermittent light stimulation at various time intervals.

Age (days)	Time interval (mins.)	First seizure					Second seizure				
		Seizures measured (no.)	Latency (sec.)		Duration (sec.)		Seizures measured (no.)	Latency (sec.)		Duration (sec.)	
			Mean	SE	Mean	SE		Mean	SE	Mean	SE
1	0	16	21.3	1.4	64.7	11.9	9	23.2	2.9	59.0	7.8
	5	16	26.1	2.8	69.3	8.5	16	29.3	3.3	42.5	9.3
	15	16	20.8	2.0	83.7	14.8	16	23.3	2.3	66.5	18.3
	30	16	19.0	1.4	199.1	56.9	15	22.5	1.7	123.2	39.7
	60	16	20.8	1.6	71.1	21.5	16	24.9	1.8	107.3	18.2
28	0	31	26.1	1.8	42.5	2.1	1	16.0	-	29.0	-
	5	28	25.4	1.4	45.0	2.5	14	25.9	1.1	40.8	3.7
	15	30	23.8	1.7	45.7	1.8	23	26.6	1.7	44.4	3.5
	30	28	24.9	1.7	47.4	1.9	24	27.6	2.1	46.0	3.9
	60	27	24.5	1.9	44.5	2.5	24	29.3	2.3	47.1	3.0
56	0	15	21.7	2.9	45.9	3.5	0	-	-	-	-
	5	15	22.7	2.7	44.2	3.2	8	26.6	1.9	44.5	4.9
	15	15	22.9	2.4	73.9	22.3	9	24.1	2.5	46.6	3.5
	30	15	18.3	2.1	51.7	2.2	15	20.6	1.6	107.0	24.5
	60	15	20.9	2.2	61.5	13.5	15	22.9	2.6	49.5	2.9

Table 32: Analyses of variance of effects of time intervals on seizure latency and duration of the second seizure.

Age (days)	Time interval (mins.)	Source of variation	df	Seizure latency		Seizure duration	
				Mean square	F	Mean square	F
	0	Seizure order	1	21.00	0.46	186.32	0.11
		Error	23	45.43		1678.32	
	5	Seizure order	1	81.28	0.53	5751.28	4.81 *
		Error	30	152.37		1195.48	
1	15	Seizure order	1	47.53	0.65	2288.18	0.52
		Error	30	72.91		4433.25	
	30	Seizure order	1	96.66	2.54	44629.20	1.17
		Error	29	38.06		38170.69	
	60	Seizure order	1	140.28	3.05	87.78	0.01
		Error	30	45.93		6350.45	
	0	Seizure order		-	-	-	-
		Error		-		-	
	5	Seizure order	1	3.04	0.07	162.97	0.91
		Error	40	44.93		178.83	
28	15	Seizure order	1	101.96	1.32	111.38	0.63
		Error	51	77.16		177.45	
	30	Seizure order	1	93.55	1.05	25.07	0.11
		Error	50	89.21		220.77	
	60	Seizure order	1	294.56	2.66	83.59	0.43
		Error	49	110.57		193.97	
	0	Seizure order		-	-	-	-
		Error		-		-	
	5	Seizure order	1	81.76	1.02	0.47	0.003
		Error	21	79.91		166.31	
56	15	Seizure order	1	8.71	0.12	4216.18	0.88
		Error	22	74.30		4765.96	
	30	Seizure order	1	38.54	0.74	22908.04	5.05 *
		Error	28	52.25		4535.39	
	60	Seizure order	1	32.04	0.36	1080.01	0.75
		Error	28	89.52		1431.91	

* $P < 0.05$.

Table 33: Latency and duration of successive seizures of epileptic chickens subjected to intermittent light stimulation (14 fps) with various time intervals at four weeks of age.

Birds tested (band no.)		Sequence of tests at 15-minute intervals								Birds tested (band no.)		Sequence of tests at 30-minute intervals							
		1st	2nd	3rd	4th	5th	6th	7th	8th			1st	2nd	3rd	4th	5th	6th	7th	8th
71-1037	L	17	40	44	28	52	-	-	-	71-0246	L	28	63	40	44	71	48	36	60
	S	65	48	55	30	65					S	38	20	28	42	36	39	45	33
71-1041	L	23	30	29*	29	58	-	-	-	71-0250	L	24	26	25	55	26	60	25	37
	S	55	38	10	46	180					S	31	55	40	61	53	59	65	55
71-1045	L	26	24	80*	40	42	-	-	-	71-0251	L	25	18	34	22	23	28	25	28
	S	35	44	7	28	129					S	30	30	60	67	56	43	40	48
71-1034	L	26	30	45	-	-	-	-	-	71-0258	L	23	26	17	17	17	24	24	18
	S	53	62	248							S	46	60	50	50	48	41	61	48
71-1044	L	35	36	34	-	-	-	-	-	71-0268	L	25	31	29	28	28	32	33	13
	S	64	53	78							S	33	45	57	42	38	49	40	35
71-1046	L	29	36*	50	-	-	-	-	-	Average **	L	25	33	29	33	33	38	29	31
	S	40	20	30							S	36	42	47	52	46	46	50	44
71-1039	L	24	25*	-	-	-	-	-	-										
	S	32	50																
71-1036	L	28	-	-	-	-	-	-	-										
	S	25																	
71-1047	L	27	-	-	-	-	-	-	-										
	S	30																	
71-1068	L	-	-	-	-	-	-	-	-										
	S																		
Average **	L	26	32	43	32	51	-	-	-										
	S	44	49	103	35	125													

L = Latency in seconds.
S = Seizure duration in seconds.
* = Incomplete seizure.
**= Data for incomplete seizures were not included.

Table 34: Latency and duration of successive seizures of epileptic chickens subjected to intermittent light stimulation (14 fps) with various time intervals at eight weeks of age.

Birds tested (band no.)		Sequence of tests at 5-minute intervals							
		1st	2nd	3rd	4th	5th	6th	7th	8th
71-0265	L	31	24	19	18	24	31*	-	-
	S	45	30	32	35	78	30		
71-0254	L	23	32	31	55*	26*	-	-	-
	S	50	42	58	10	20			
71-0242	L	24	31	-	-	-	-	-	-
	S	25	21						
71-0266	L	16	21	-	-	-	-	-	-
	S	43	1127						
71-0267	L	17	28	-	-	-	-	-	-
	S	40	466						
71-0269	L	16	15*	-	-	-	-	-	-
	S	98	45						
71-0256	L	21	-	-	-	-	-	-	-
	S	48							
71-0264	L	18	-	-	-	-	-	-	-
	S	55							
71-0270	L	-	-	-	-	-	-	-	-
	S								
71-0262	L	-	-	-	-	-	-	-	-
	S								
Average **	L	21	27	25	18	24	-	-	-
	S	51	337	45	35	78			

Birds tested (band no.)		Sequence of tests at 15-minute intervals							
		1st	2nd	3rd	4th	5th	6th	7th	8th
71-0246	L	19	26	23	25	21	23	33	28
	S	45	50	58	98	55	47	52	60
71-0250	L	13	20	29	33	22	49	68	28*
	S	50	48	54	57	55	44	28	28
71-0251	L	21	19	22	22	39	28	35*	68
	S	43	47	55	48	118	195	18	61
71-0268	L	26	27	35	40	34	86	45	-
	S	47	46	50	55	58	30	54	
71-0258	L	13	27	26	30	40	-	-	-
	S	48	40	30	55	38			
Average **	L	18	24	27	30	31	47	49	48
	S	47	46	49	63	65	79	45	61

L = Latency in seconds.
S = Seizure duration in seconds.
* = Incomplete seizure.
**= Data for incomplete seizures were not included.

Table 35: Distribution of epileptiform seizure mutant in progeny of different parental crosses, combined data of 12 hatches.

Parental cross Male x Female		Normal chicks (no.)	Epileptic chicks (no.)	Chi-square: Sum of 12 hatches (df = 12) χ^2	P	Pooled (df = 1) χ^2	P	Heterogeneity (df = 11) χ^2	P
E x E	Observed	0	218						
	Expected	0	218	-		-		-	
E x C	Observed	66	51						
	Expected	58.5	58.5	12.39	<0.50	1.92	<0.25	10.47	<0.50
C x E	Observed	97	93						
	Expected	95	95	1.81	<1.00	0.08	<0.90	1.73	<1.00
C x C	Observed	164	53						
	Expected	162.8	54.2	8.99	<0.75	0.04	<0.90	8.95	<0.75
N x E	Observed	98	0						
	Expected	98	0	-		-		-	
N x N	Observed	75	0						
	Expected	75	0	-		-		-	

E = Epileptic; C = Carrier; N = Normal.

Table 36: Sex distribution in chicks obtained from different parental crosses, combined data of 12 hatches.

Parental cross Male x Female	Phenotype		Sex distribution Male	Female	Chi-square: Sum of 12 hatches (df = 12) χ^2	P	Pooled (df = 1) χ^2	P	Heterogeneity (df = 11) χ^2	P
E x E	Normal	Obs	0	0	-	-	-	-	-	-
		Exp	0	0						
	Epileptic	Obs	110	108	10.44	<0.75	0.02	<0.90	10.42	<0.50
		Exp	109	109						
E x C	Normal	Obs	32	34	10.10	<0.75	0.06	<0.90	10.04	<0.75
		Exp	33	33						
	Epileptic	Obs	25	26	16.51	<0.25	0.02	<0.90	16.49	<0.25
		Exp	25.5	25.5						
C x E	Normal	Obs	48	49	7.17	<0.90	0.01	<0.95	7.16	<0.90
		Exp	48.5	48.5						
	Epileptic	Obs	47	46	10.29	<0.75	0.01	<0.95	10.28	<0.75
		Exp	46.5	46.5						
C x C	Normal	Obs	89	75	8.66	<0.75	1.15	<0.50	7.51	<0.90
		Exp	82	82						
	Epileptic	Obs	29	24	7.35	<0.90	0.47	<0.50	6.88	<0.90
		Exp	26.5	26.5						
N x E	Normal	Obs	53	45	14.01	<0.50	0.65	<0.50	13.36	<0.50
		Exp	49	49						
	Epileptic	Obs	0	0	-	-	-	-	-	-
		Exp	0	0						
N x N	Normal	Obs	39	36	12.39	<0.50	1.92	<0.25	10.47	<0.50
		Exp	37.5	37.5						
	Epileptic	Obs	0	0	-	-	-	-	-	-
		Exp	0	0						

E = Epileptic; C = Carrier; N = Normal.
Obs = Observed value; Exp = Expected value.

Table 37: Fertility, embryonic mortality and hatchability of eggs obtained from matings involving epileptic and non-epileptic chickens.

Birds used (no. and genotype)	Data including eggs with inferior shell quality							Data excluding eggs with inferior shell quality						
Male x Female	Eggs set (no.)	Fertile eggs (no.)	(%)	Embryonic mortality (no.)	(%)	Hatchability of fertile eggs (no.)	(%)	Eggs set (no.)	Fertile eggs (no.)	(%)	Embryonic mortality (no.)	(%)	Hatchability of fertile eggs (no.)	(%)
5 E x 8 E	283	262	92.3	44	16.8	218	83.2	283	262	92.3	44	16.8	218	83.2
5 E x 8 C	177	163	92.1	46	28.2	117	71.8	155	146	94.2	32	21.9	114	78.1
5 C x 8 E	256	231	90.2	41	17.7	190	82.3	256	231	90.2	41	17.7	190	82.3
5 C x 19 C	345	300	87.0	83	27.7	217	72.3	286	254	88.8	49	19.3	205	80.7
5 N x 5 E	144	135	93.8	37	27.4	98	72.6	83	75	90.4	16	21.3	59	78.7
5 N x 6 N	107	99	92.5	24	24.2	75	75.8	75	68	90.7	14	20.6	54	79.4
χ^2 (df = 5)			2.21		53.09		324.73			0.15		2.37		1.86
Probability			<0.90		<0.01		<0.01			<1.00		<0.90		<0.90

E = Epileptic; C = Carrier; N = Normal.

Table 38: Characteristics of EEG patterns recorded from 12 epileptic, five epilepsy carrier, and four normal chickens between three and four months of age.

Genotype	Lead*	Resting EEG				EEG during ILS (14 fps)			
		Average frequency of waves (cps)	Frequency range (cps)	Average amplitude (μv)	Amplitude range (μv)	Average frequency of waves (cps)	Frequency range (cps)	Average amplitude (μv)	Amplitude range (μv)
Epileptic	A	1.7	1.2 - 2.4	121	72 - 190	14	-	106	33 - 237
	B	1.6	1.2 - 1.8	115	47 - 216	14	-	137	36 - 263
	C	1.6	1.0 - 2.0	135	76 - 177	14	-	113	36 - 167
	D	1.6	1.2 - 2.0	114	72 - 139	14	-	128	76 - 221
	E	1.6	1.0 - 2.2	150	81 - 187	14	-	112	48 - 208
	F	1.6	1.2 - 2.0	194	107 - 279	14	-	133	58 - 252
	G	1.7	1.0 - 2.4	193	89 - 248	14	-	85	49 - 155
Epilepsy carrier	A	2.9	2.2 - 3.6	35	21 - 55	3.2	2.2 - 4.0	30	19 - 51
	B	2.8	2.4 - 3.8	34	21 - 52	3.6	2.4 - 6.6	34	22 - 64
	C	3.9	3.0 - 6.3	42	35 - 54	3.9	3.0 - 6.2	43	22 - 79
	D	3.4	3.0 - 3.6	43	25 - 63	3.8	3.0 - 4.6	39	14 - 78
	E	3.3	2.4 - 4.6	61	39 - 73	3.6	3.0 - 6.0	59	32 - 123
	F	3.6	2.8 - 5.8	61	19 - 118	4.0	2.8 - 7.0	67	32 - 173
	G	3.3	2.8 - 3.8	116	72 - 144	2.6	2.0 - 4.0	116	100 - 128
Normal	A	3.7	3.2 - 4.4	38	32 - 44	3.9	3.6 - 4.2	44	38 - 57
	B	3.9	3.2 - 4.4	39	21 - 64	3.8	3.2 - 4.8	44	29 - 68
	C	3.8	3.2 - 4.2	38	27 - 54	3.9	3.4 - 4.4	47	32 - 70
	D	3.4	3.0 - 3.6	40	31 - 46	3.4	2.4 - 4.2	45	23 - 65
	E	3.2	2.8 - 3.6	56	38 - 67	3.2	3.0 - 3.4	62	51 - 71
	F	3.1	2.6 - 3.4	90	38 - 114	3.2	3.0 - 3.4	100	41 - 129
	G	3.3	3.0 - 3.6	114	75 - 159	3.6	2.6 - 5.0	127	93 - 185

* A specific pair of electrodes used in recording the potential differences between two locations on the surface of the chicken's skull (see Figure 1).

Table 39: Estimated t values for the comparison of average frequency and average amplitude brainwaves of epileptic, carrier and normal chickens.

	Lead	Resting EEG			EEG during ILS (14 fps)	Resting EEG vs. EEG during ILS (14 fps)	
		Epileptic vs. carrier (df = 15)	Epileptic vs. normal (df = 14)	Carrier vs. normal (df = 7)	Carrier vs. normal (df = 7)	Carrier chickens (df = 8)	Normal chickens (df = 6)
Average frequency	A	-5.21 **	-8.34 **	-2.07 *	-1.93 *	-0.72	-0.66
	B	-6.44 **	-12.48 **	-2.77 *	-0.21	-0.93	0.00
	C	-5.75 **	-10.63 **	-0.20	0.03	-0.02	-0.45
	D	-12.62 **	-12.64 **	-0.40	0.85	-1.56	0.11
	E	-6.28 **	-8.41 **	0.27	0.73	-0.46	0.26
	F	-5.49 **	-7.97 **	0.90	0.84	-0.33	-0.79
	G	-5.51 **	-6.99 **	0.06	-1.33	1.31	-0.56
Average amplitude	A	4.74 **	4.16 **	-0.39	-1.75	-0.54	1.03
	B	3.41 **	2.80 **	-0.59	-0.87	0.08	0.40
	C	6.01 **	5.53 **	0.56	-0.35	-0.05	-0.89
	D	4.24 **	4.09 **	0.31	-0.46	0.30	-0.57
	E	4.52 **	4.53 **	0.35	-0.12	0.07	-0.76
	F	4.59 **	3.55 **	-0.79	-0.91	0.09	-0.38
	G	2.49 *	2.85 **	0.06	-0.42	-0.01	-0.45

* $P < 0.05$; ** $P < 0.01$.

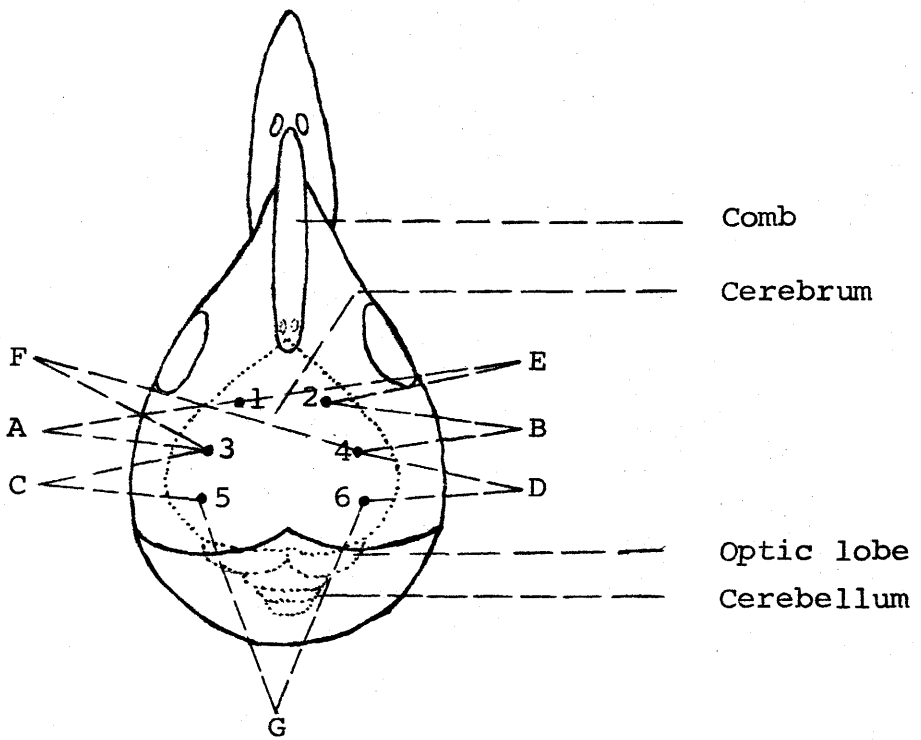


Fig. 1: A dorsal view of the chicken skull and the locations of the electrodes. The numbers (1,2,3,4,5, and 6) represent the locations of the electrodes implanted. Dotted lines show the approximate outline of the brain. The capital letters (A,B,C,D,E,F, and G) represent the recording leads connected in bipolar method.

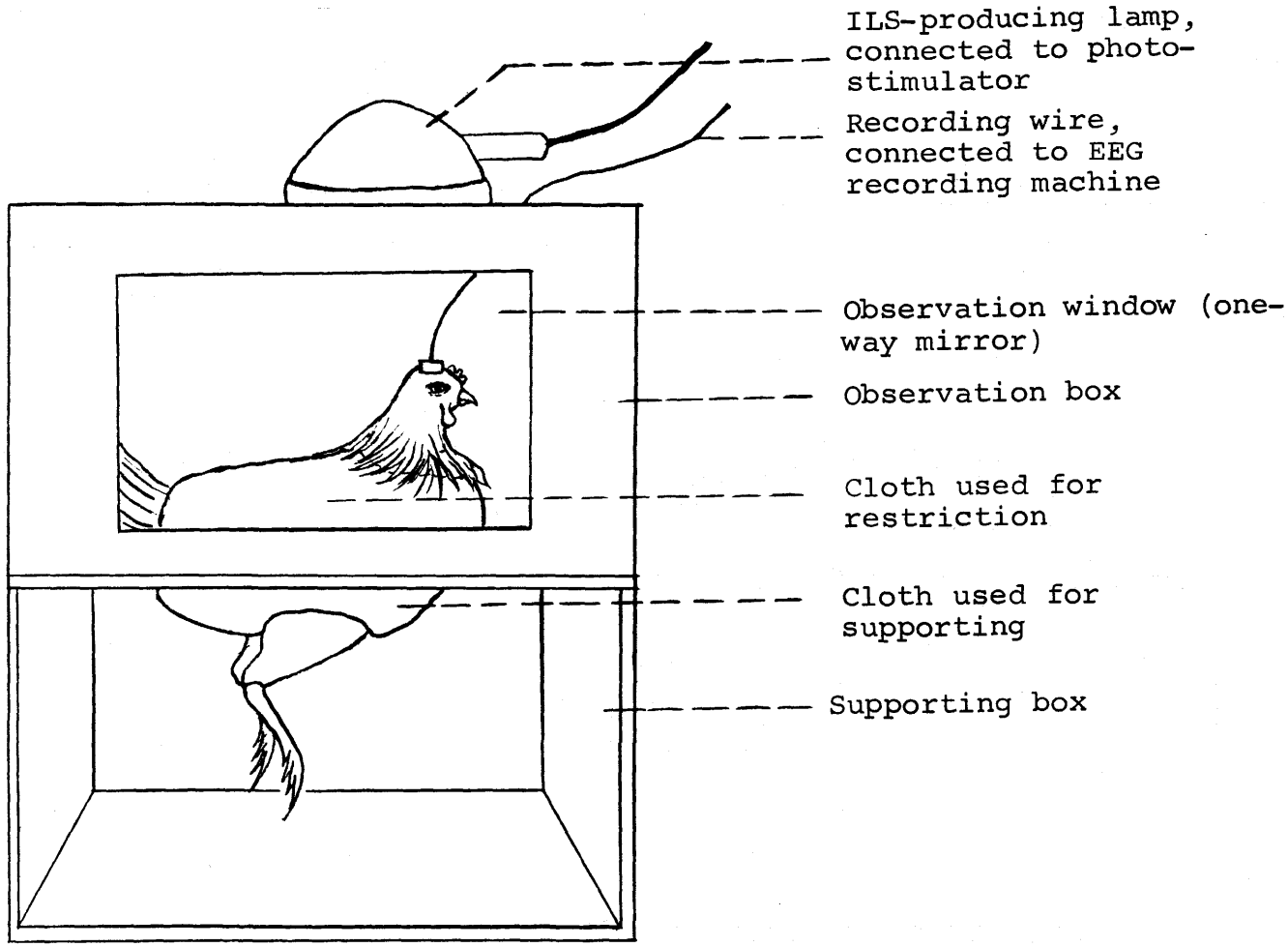


Figure 2: Diagram of the EEG recording apparatus.

Figure 3

Figure 3: The typical pattern of complete epileptiform seizures in chickens induced by intermittent light stimulation. The pictures show a sequential response of an 11-day old chick to intermittent light stimulation of 14 fps.

A: The bird begins to respond to the stimulus with head turning upwards.

B: Head turns to the side; the chick is pecking with its beak.

C: Head turns upwards and sideways.

D and E: The chick falls to the floor and begins convulsive movements.

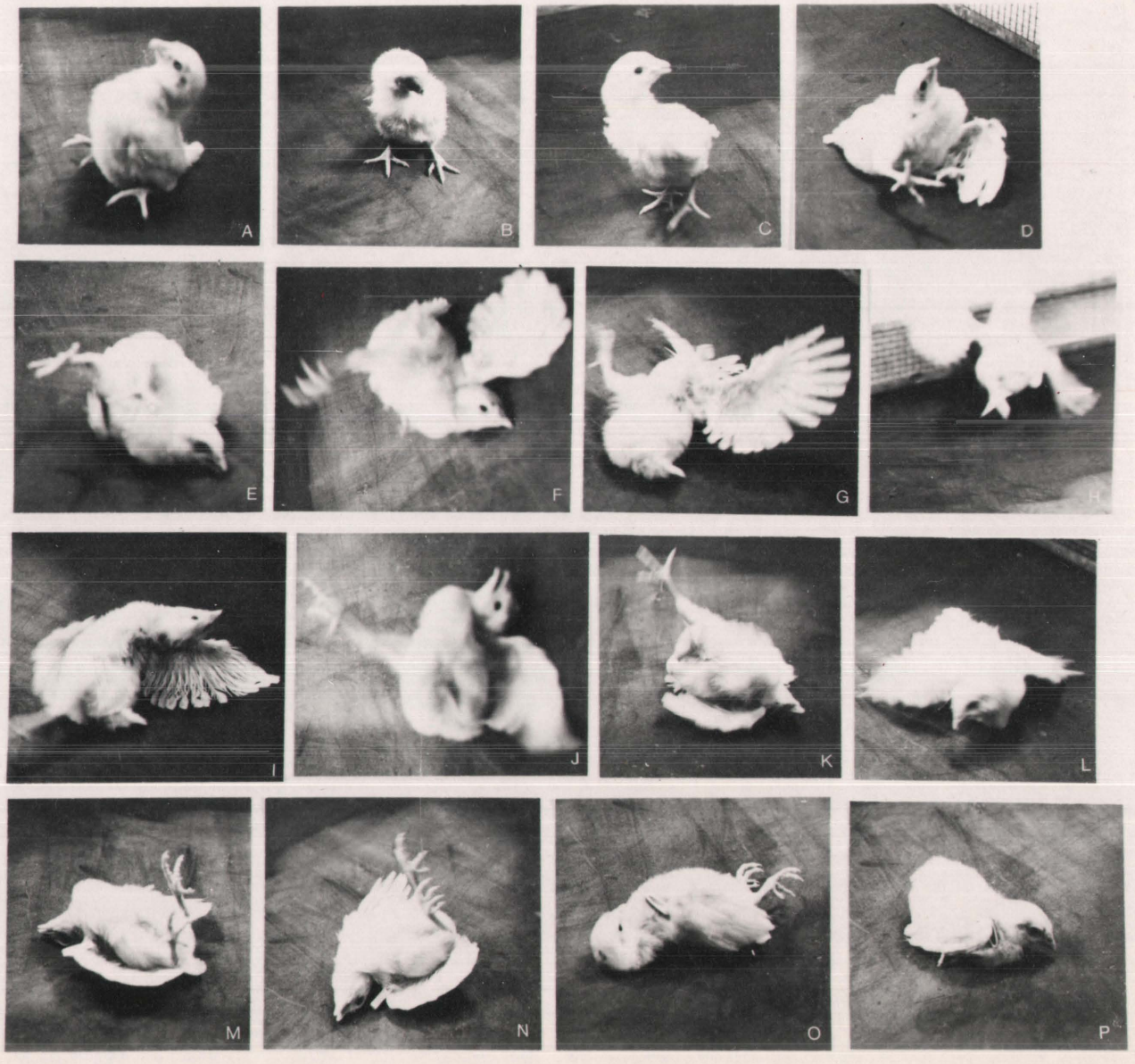
F and G: Severe clonic convulsive movements with legs thrashing violently and wings stretching out and flapping.

H: The chick suddenly gets up and dashes in any direction.

I,J,K and L: The chick falls back to the floor and continues clonic convulsions.

M,N and O: The convulsive movements begin to slow down; the seizure is close to the end.

P: After the seizure, the chick remains quiet and motionless for several seconds or more.


A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P

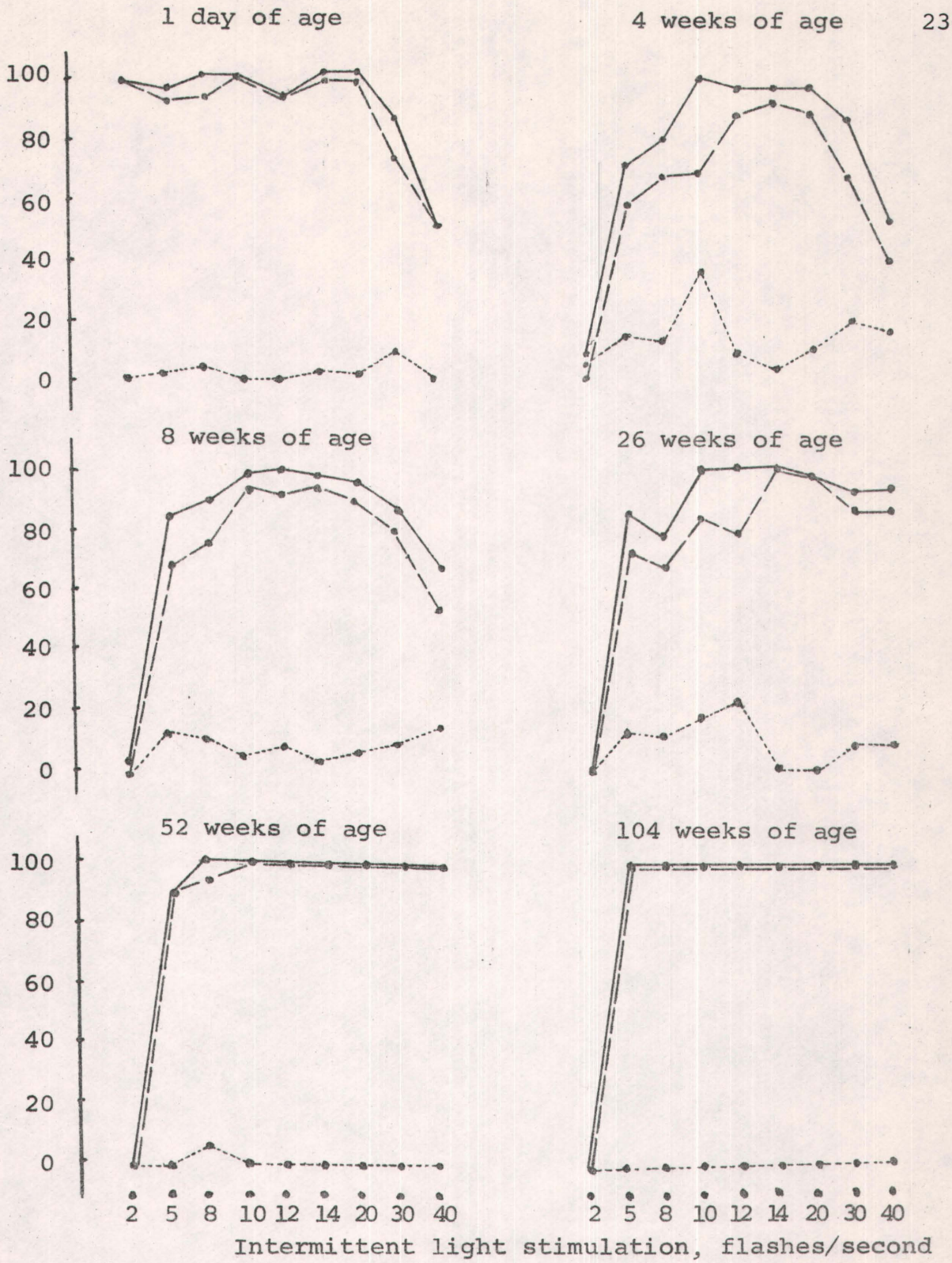


Figure 4: Seizure susceptibility (% of birds responding) and seizure severity (complete and incomplete seizures) of epileptic chickens subjected to intermittent light stimulation at various flash frequencies at different ages. —— = seizure susceptibility, ---- = complete seizure, ... = incomplete seizure.

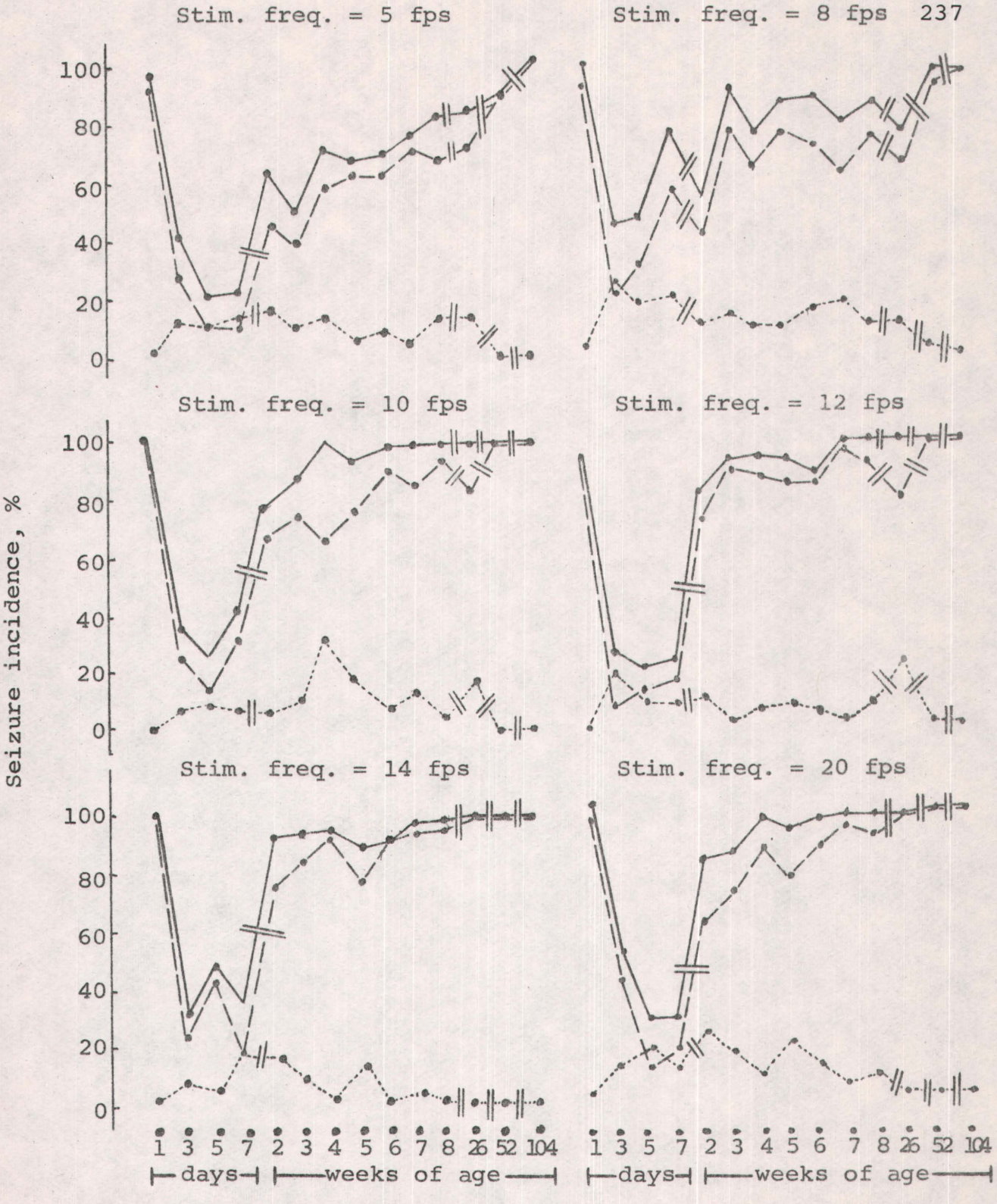


Figure 5: Effect of age on seizure susceptibility (% of birds responding) and seizure severity (complete and incomplete seizures) of epileptic chickens subjected to intermittent light stimulation. —— = seizure susceptibility, ---- = complete seizure, ... = incomplete seizure.

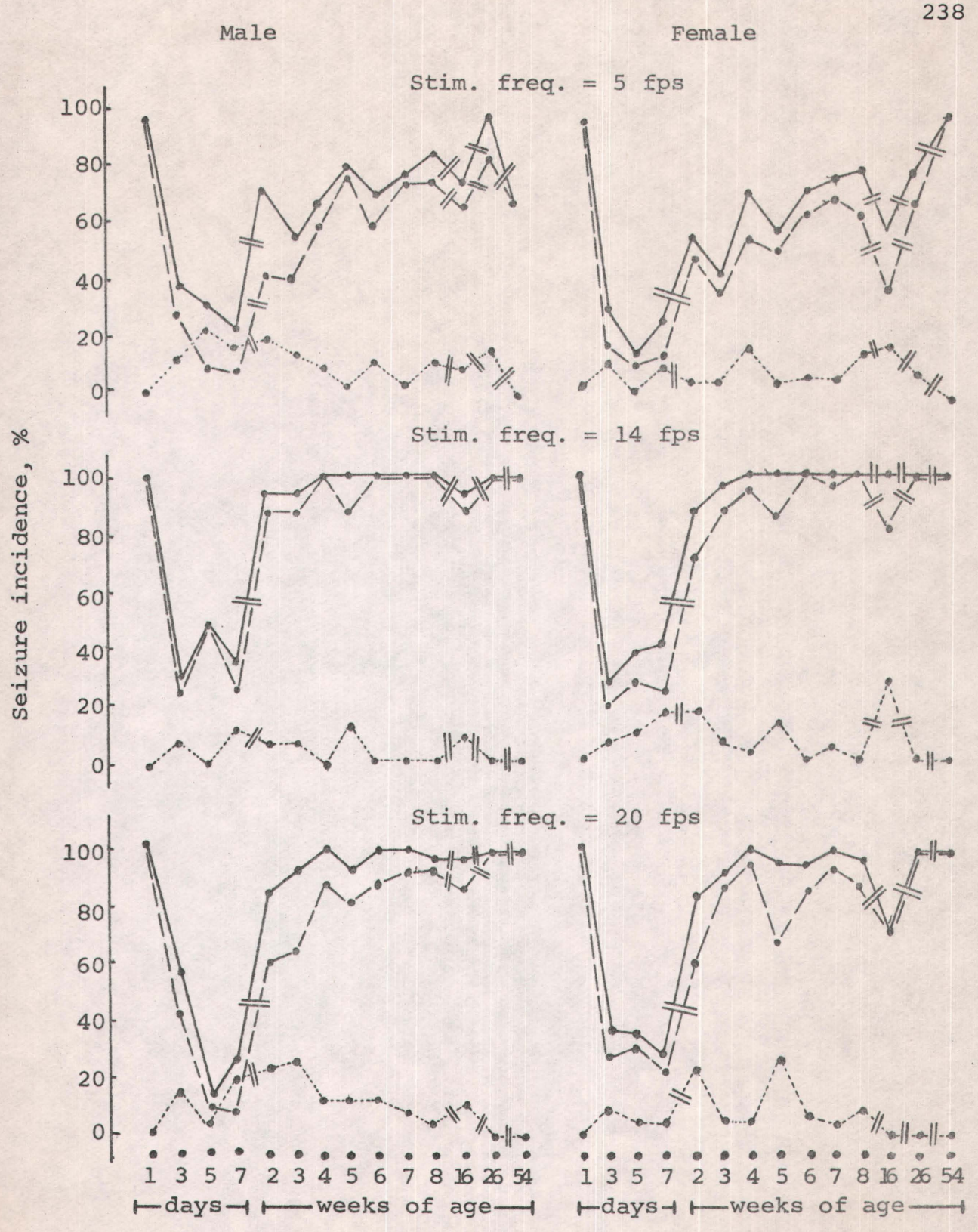


Figure 6: Seizure susceptibility (% of birds responding) and seizure severity (complete and incomplete seizures) of male and female epileptic chickens subjected to intermittent light stimulation. —— = seizure susceptibility, ---- = complete seizure, ... = incomplete seizure.

Figure 7

Figure 7: EEG patterns of chickens before and during intermittent light stimulation at a frequency of 14 fps. The numbers (1,2,3,4,5 and 6) represent the locations of the electrodes; the recording leads were connected in bipolar method (see Figure 1). Calibrations: 100 μv and 1 second.

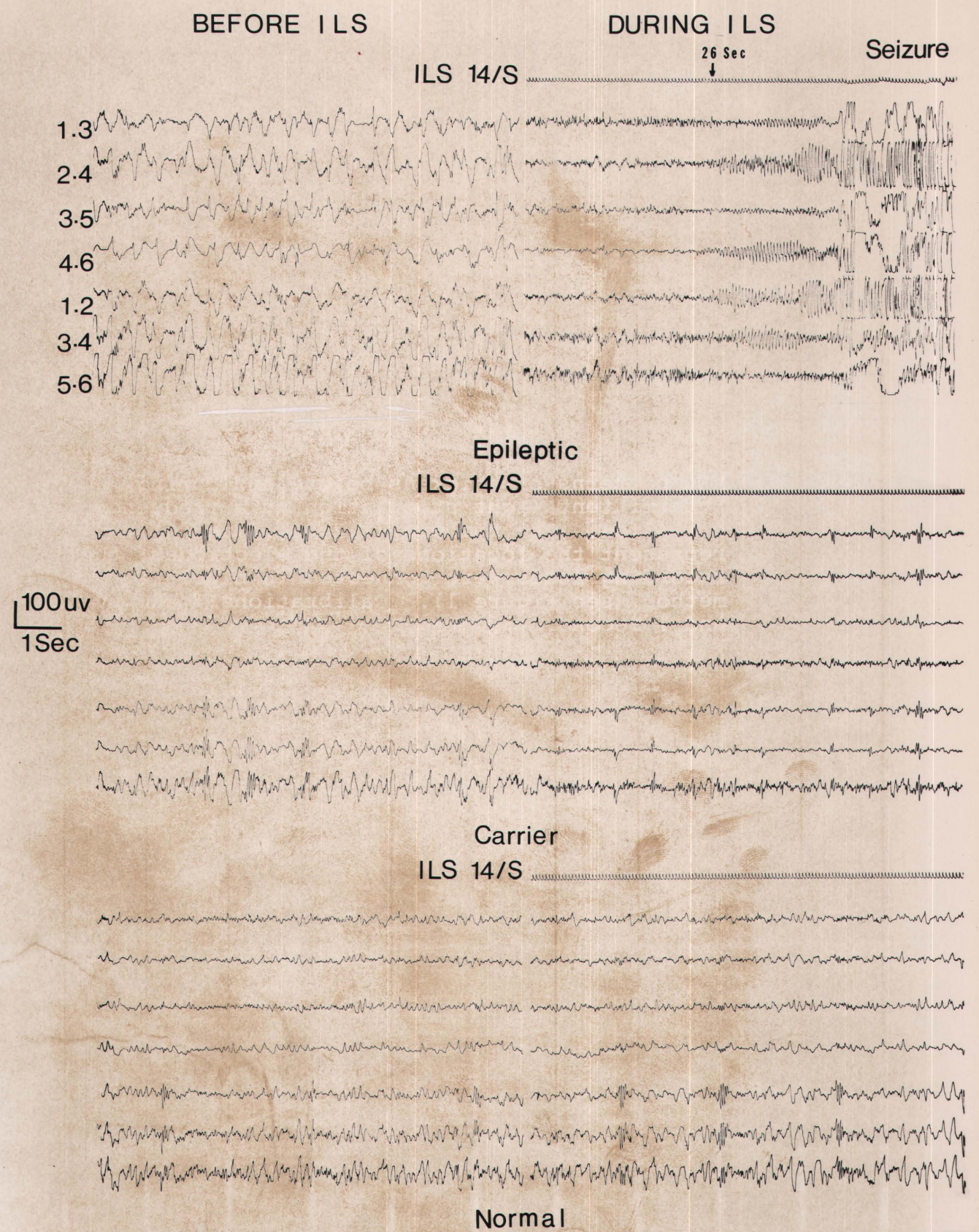

BEFORE ILS
DURING ILS
26 Sec
Seizure
ILS 14/S
1·3
2·4
3·5
4·6
1·2
3·4
5·6
Epileptic
ILS 14/S
100uv
1Sec
Carrier
ILS 14/S
Normal

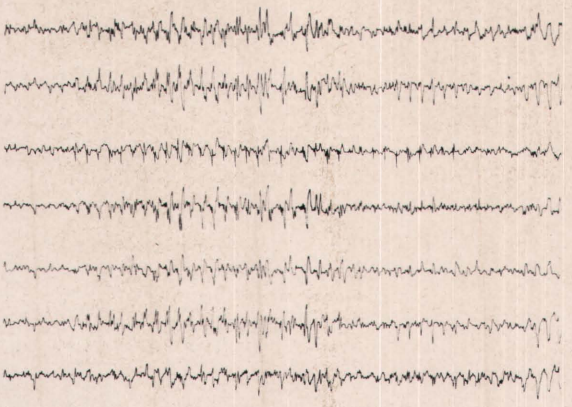
Figure 8

Figure 8: Abnormal spiking patterns of epileptic chickens under different frequencies of intermittent light stimulation. The numbers (1,2,3,4,5 and 6) represent the locations of the electrodes; the recording leads were connected in bipolar method (see Figure 1). Calibrations: 100 μv and 1 second.

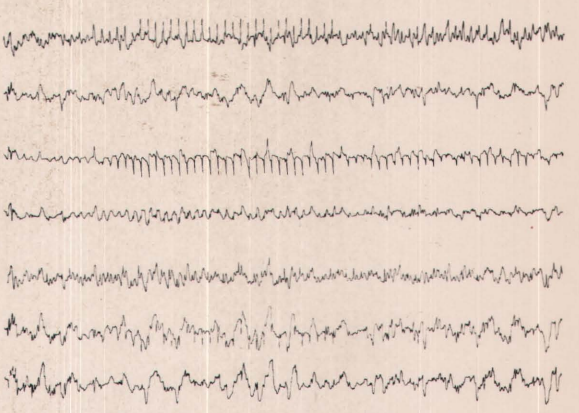
100uv
1 Sec

ILS 5/S

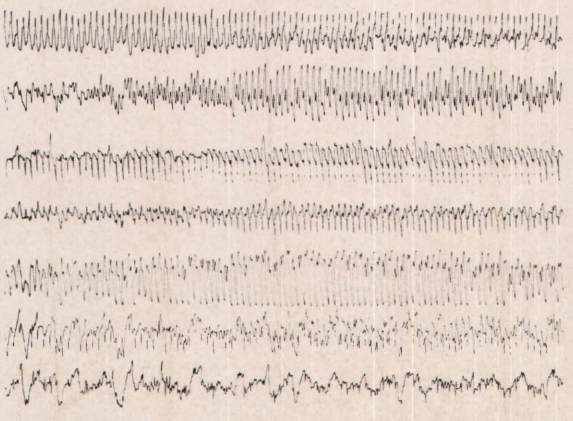
1·3
2·4
3·5
4·6
1·2
3·4
5·6



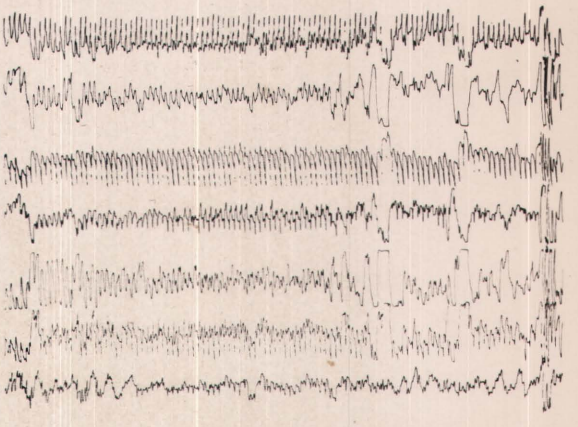
ILS 8/S



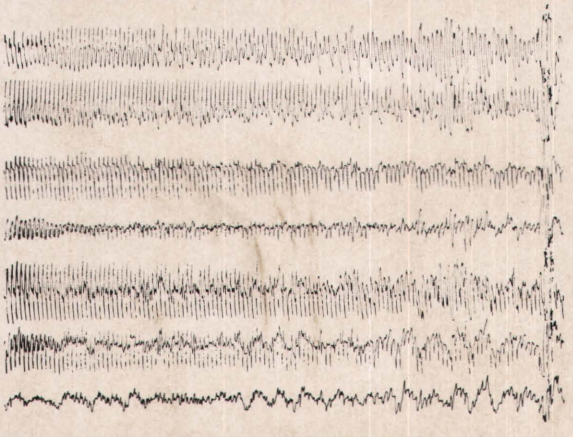
ILS 10/S



ILS 12/S



ILS 14/S



ILS 20/S